NREL/AMOCO CRADA

PHASE 3 REPORT

BACKGROUND

The Midwest Research Institute ("MRI"), the operator the National Renewable Energy Laboratory ("NREL") for the U.S. Department of Energy ("DOE") and SWAN Biomass Company, a partnership between Amoco Ethanol Development Corporation and Stone and Webster Engineering Industrial Technology Corporation, joined in a cooperative research and development agreement ("CRADA") effort from 1991 to 1996 to develop enough information at the pilot scale to justify constructing a demonstration facility for converting biomass to ethanol. This "NREL/Amoco CRADA Phase 3 Report (the "Report")" covers Phase 3 of the CRADA, from 1994 to 1996, focusing on corn stover as the feedstock, rather than waste paper as originally envisioned. This is the full report on Phase 3, but does not yet include any of a large number of appendices. As many of these as possible will be posted later.

Under the terms of the CRADA, information developed during the CRADA was protected from public disclosure for a period of five years. That protection period is now over, so we are making this report publicly available. The CRADA, however, also comprises certain Amoco proprietary processes. Information about those processes continues to be protected. Such information has been deleted from the Report. Unfortunately, the Report is not available in original electronic format, so you may find blank spaces where Amoco proprietary information was deleted and some unintended font changes and other typography mistakes.

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NREL-Amoco CRADA
Phase 3 Report

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Executive Summary

The National Renewable Energy Laboratory (NREL) and SWAN Biomass Company, a partnership between Amoco Ethanol Development Corporation and Stone and Webster Engineering Industrial Technology Corporation, have completed a work plan developed in August, 1995 for Phase 3 of the Amoco/NREL Cooperative Research and Development Agreement (CRADA) 9 I-0003. The objective of Phase 3 was to develop sufficient information at pilot plant scale to justify construction of a demonstration facility for converting biomass to ethanol. Phase 3 included a laboratory program to evaluate the yeast strains developed at Purdue University and support pilot scale testing, a series runs in the NREL process development unit (PDU) using corn fiber as the feedstock, and process design and economic modeling.

Significant progress was made in the definition and verification of the biomass to ethanol process. However, a number of technical problems still need to be solved, and a fully integrated run demonstrating the proposed commercial process configuration in the PDU must be made before construction of a demonstration facility is justified. Most of the technical problems can be addressed in tests using any of a number of feedstocks. The most significant unresolved generic problem is how to eliminate or control the impact of acetic acid on xylose fermentation. For corn fiber feedstock, it is also critical to learn how to collect and dry the solid coproduct.

The estimated cost for ethanol produced from corn fiber in a commercial facility is about $0.86 per gallon, including a 15% rate of return on the capital. Failure to satisfactorily resolve the technical issues uncovered during Phase 3 could raise that cost substantially.

Summary Results

Laboratory Program

The key objective of the laboratory program was to develop bench scale data on the performance of fermenting organisms before they were used in the PDU. Work was limited to the evaluation of three yeasts acquired from Purdue University, *Saccharomyces* Strain 1400 and two recombinant derivatives: 1400 (pLNH33), a yeast with xylose-fermenting genes in an inserted plasmid, and 1400 (LNHST2), with xylose-processing genes integrated into the yeast chromosome. Experimentation included growth studies, yeast addition rates, and the impact of inhibitors, nutrients, and solids levels.

Continuous and batch studies demonstrated that 1400 (LNHST2) can metabolize xylose more effectively than 1400 (pLNH33), particularly in the presence of glucose. Furthermore, the latter was completely stable in all tests run, even when xylose is not present to maintain pressure on the organism to retain the xylose-conversion genes. Acetic acid and lactic acid were shown to inhibit xylose utilization. High concentrations of furfural and HMF seem to cause a lag phase observed in batch SSCF.

SSCF Kinetic Model

Reaction kinetics modeling was used in parallel with all bench scale and PDU experiments as a guide to subsequent work. The SSCF mode1 includes expressions describing cellulose hydrolysis to both cellobiose and glucose, cellobiose hydrolysis to glucose, glucose and xylose fermentation, and furfural and HMF disappearance. The model accurately predicts batch fermentation behavior in both shake flasks and larger fermenters, and predicted concentrations within 6% of those measured during PDU runs. However, the mode1

parameters were determined by fitting the equations to data where individual effects were not isolated. Ideally one would like to design and conduct experiments that isolated variables to determine model parameters and then test the model on data from more complex experiments. Because of the way the model was developed, it fits the existing data, but there is some uncertainty that it can be used to extrapolate to other conditions or feedstocks.

PDU Operations

The PDU includes equipment for feedstock handling, size reduction, pretreatment, fermentation (3 stages), distillation and solid-liquid separation. It is supported by utilities for steam, cooling water, chilled water, hot water, deionized water, plant and instrument air, and nitrogen. The nominal maximum feed rate is 1 ton per day of dry biomass.

Seven runs were made in the PDU. The first four used Strain 1400 and SSF, the last three used the recombinant yeast, LNHST2, and SSCF. The runs with 1400 were useful to demonstrate PDU operability over the range of parameters of interest for the CRADA program, but did not provide relevant information on fermentation performance.

Amoco Pretreatment Reactor (APR) Results

The development of the APR has been one of the key accomplishments in Phase 3. Amoco had conceived and tested the APR at low throughput in their joint development program with Canada. The Phase 3 program confirmed that this device was capable of supplying high-quality pretreated feed for SSCF with direct steam injection to supply process heat, and showed that it was possible to increase the flowrate through the APR by at least 8 times. However, the program failed to determine optimum operating conditions for corn fiber at high throughput, and mass balances were never closed satisfactorily around the APR.

The APR performed through four continuous runs of 224, 695, 1100 and 977 hours in Phase 3. As mechanical problems were resolved, downtime decreased to about 5%; downtime should virtually disappear with the implementation of operational improvements.

Fermentation Results

Batch fermentations performed using LNHST2 at the bench scale and in the PDU showed no performance differences with increased reactor size. Bench-scale continuous fermentations also had similar performance to PDU runs. Thus, bench scale runs are a good indication of large scale performance.

The last two PDU runs, each about 1000 hours long, provided continuous performance information on SSCF using LNHST2. During these fermentations, all of the monomeric and most the oligomeric glucose in the

pretreated material was converted to ethanol and other products. However, while xylose conversion was about 75% at 15% solids in the feed to the first fermenter, at 25% solids xylose conversion fell to 25%. Poor xylose conversion was caused by acetic acid, which is an unavoidable byproduct of pretreatment, and proportional to solids level fed to SSCF.

The ethanol process yields (defined as ethanol produced divided by potential ethanol from starch, cellulose, galactan, and xylan) for PDU runs ranged from 40% to 55%. These low yields are due to inadequate pretreatment, acetic acid inhibition of xylose fermentation, and sometimes lower than expected conversion of glucose oligomers to fermentable form. More sugars must be converted to ethanol to make the process economically attractive.

Contamination was a recurring problem during PDU runs. *Lactobacillus* bacteria were routinely found in the fermenters, and isolated in CSL and in pretreated feed when there were problems with pretreatment equipment operation.

Solid Residue Recovery and Testing Results

Ten tons (dry basis) of solid coproduct was produced during the last two PDU runs for testing the solid coproduct as an animal feed supplement. The processing requirements for separating the solids from the liquids in the bottoms product from the distillation column and making a marketable product were also examined. The techniques tested for solid-liquid separation were not attractive for commercial use.

Centrifuge tests, conducted at NREL and by two different vendors, indicated that there is a significant tradeoff between high percent solids in the wet cake and solids recovery levels, and that decanter centrifuges may not be an appropriate choice for commercial facilities. Vendor tests also suggested that rotary vacuum filtration is not practical for this application. Coproduct drying tests were carried out in a rotary steam tube dryer in a vendor shop. Results indicate that although this type of equipment can be used, the cake is so wet that very large solids recycle is needed, and repeated heating downgrades the product by darkening it and possibly reducing its nutritional value. Drying the material in this manner results in a fine powder which must be subsequently pelletized before it can be used in the animal feed market.

Animal feeding tests were carried out on poultry, swine and cattle. Final test results are not available yet, but will be distributed as an addendum to this report when they are received from the subcontractors.

Process Design

A conceptual process design and technoeconomic evaluation was carried out in order to evaluate the commercial application of the SWAN biomass-to-ethanol technology to a feedstock blend of corn fiber and corn screenings. It was assumed that the feedstock would be provided by a corn wet mill in Pekin, Illinois (approximate capacity of 150,000 bushels of corn per day) with the biomass-to-ethanol unit installed in an integrated fashion with the mill and assumed to operate 350 days per year, 24 hours per day.

A platform case was defined and used as the basis for complete process flow diagrams and a detailed costed equipment list. The plant design includes feedstock transfer from the wet mill, pretreatment, neutralization, fermentation, ethanol recovery and dehydration, ethanol blending and storage, animal feed coproduct processing and storage, chemical storage, and a chilled water system. Other utility and support systems

required for the plant's operation is assumed to be provided by the wet mill plant. They are accounted for through operating costs and other assessments.

The platform case facility is fed 750 dry tons per day of corn fiber and corn screenings per day, and produces 21.4 million gallons per year of ethanol.

Technoeconomic Evaluation

The economic evaluation uses a discounted cash flow analysis, and fourth quarter 1996 dollars. The result of the analysis is given as the cost to produce a gallon of ethanol, including a 15% internal rate of return on invested capital. There is no inflation, and 100% equity financing.

A spreadsheet model was developed and used to estimate ethanol cost. After setting input parameters, the model is run in an iterative fashion varying enzyme dose, SSCF residence time, internal recycle rates and other parameters, until the lowest cost for ethanol product is found. Yields and conversions used in the optimization are determined separately in the SSCF kinetic model.

The capital cost was estimated to be $41 .0 million, +/- 25%. The major capital equipment costs are based on vendor quotes or internal SWEC information, and the installed cost is factored from this information. The total capital cost includes 4% construction management, 8% for engineering design, 2% for procurement, 3% freight on equipment, 3% sales tax on equipment and 20% contingency.

The cost of ethanol from this facility was estimated to be $0.86/gal. Ethanol costs are made up of three major pieces, variable operating costs, fixed operating costs, and byproducts credits. Variable operating costs are those for feedstock ($0.38/gal), utilities ($0.16/gal), chemicals ($0.14/gal) and enzymes ($0.18/gal). Fixed operating costs consist of the labor ($0.06/gal), MTIO ($0.03/gal) and capital costs ($0.34/gal). There are byproducts credits for methane ($0.05/gal) and animal feed coproduct ($0.38/gal). The animal feed value is the same as the feed cost because both are based on an invariant value for protein, and almost all protein is recovered in the solid product.

Sensitivity Studies

During Phase 3 several design and operating parameters were identified as having potentially significant effects on the capital and operating costs for the conceptual design. A total of thirty seven economic sensitivity studies were carried out using the spreadsheet model to identify any work needed to justify the CRADA proceeding to Phase 4. Both kinetics and operating variables were modified to examine their incremental effect on the cost of ethanol from the platform case design. All parameters except those under consideration were held constant. Each run was optimized for residence time and cellulase dosage.

The study of sensitivities was lopsided in that many more of the effects examined had a negative impact on costs than a positive one. This is not uncommon when new processes are evaluated because it is generally easier to see what can go wrong than to envision reasonable improvements. Most improvements are already included in the platform case.

The sensitivity studies showed that the following must be demonstrated before it is prudent to proceed to Phase 4 under the CRADA:

a. Cost-effective removal of acetic acid on some biomass feedstock, in the PDU. If acetic acid can be recovered and sold, the byproduct credits could be very important to project justification.
b. Optimum pretreatment of corn fiber/corn screenings, using steam injection to avoid the problems encountered in Phase 3 PDU runs.
c. Coproduct solids can be properly dried and successfully tested as an animal food if corn fiber is the feedstock of choice, The value of the coproduct is critical to the overall economics for this process.

Other improvements could reduce the cost of ethanol, but are not critical:

a. Improve the fermentive organism to react xylose to ethanol more quickly when acetic acid (or lactic acid) and ethanol are present.
b. Discover why starch conversion to ethanol was incomplete in some PDU runs, and identify how to avoid this problem.
c. Ammonia should be proven in the PDU as a source of nitrogen for the fermentive organism. This will both reduce the cost of the nutrient and help keep lactic acid out of the SSCF vessels. It is expected that if ammonia works for any feedstock, it should work for corn fiber; the reverse may not be true, since corn fiber contains all necessary nutrients other than nitrogen.

Recommendations

The Phase 3 data and results of the process design/economic analysis lead to the conclusion that the ethanol from corn fiber process developed under the CRADA is not ready yet for scale up to a demonstration plant. The key work needed to prepare the technology for commercial demonstration includes: (1) development of technology to control the effects of acetic acid on xylose fermentation; (2) determination of optimum pretreatment parameters for a feedstock of interest; (3) testing and selection of equipment to separate product solids from waste liquids suitable for commercial use, and (4) integrated operation of the PDU using the configuration expected for the demonstration facility.

1.0 Introduction

The National Renewable Energy Laboratory (NREL) and SWAN Biomass Company, a business partnership between Amoco Corporation and Stone and Webster Engineering Corporation, have successfully completed work defined as Phase 3 of the Amoco /NREL Cooperative Research and Development Agreement (CRADA) 91-0003. The objective of Phase 3 of the CRADA was to provide sufficient information at pilot plant scale to justify construction of a demonstration facility for converting biomass to ethanol. This report thoroughly documents the work conducted toward this objective as required by the CRADA joint work statement.

1.1 Project Background

The CRADA was initiated in 1991 with the purpose of establishing a collaborative effort between NREL and Amoco to conduct research and development on a process for converting biomass feedstock into ethanol. The work was to be performed in four phases. During Phase 1, a preliminary engineering and economic analysis of a conceptual process for conversion of waste paper to ethanol was carried out. During Phase 2, laboratory work was conducted on waste paper feedstock to answer key questions and fill in gaps in data revealed in Phase 1. The results were combined with information gathered in Phase 1 to carry out a second more refined engineering and economic analysis of the proposed process. During Phase 2 of the CRADA, Amoco was involved in other work outside of the CRADA that led to the development of proprietary pretreatment technology and a proprietary yeast capable of fermenting both hexose and pentose sugars. In addition, Amoco conducted experiments on a variety of feedstocks. A combination of the uncertainties surrounding the availability of waste paper as a feedstock, and Amoco's success with pretreatment and organism development as well as with other feedstocks led to significant changes in direction for Phase 3.

1.2 Phase 3

In late 1994 the decision was made to change the CRADA feedstock from waste paper to corn fiber, and to incorporate Amoco's proprietary pretreatment process and proprietary cofermenting yeast developed at Purdue University into the Phase 3 research plan. There were three major components to the Phase 3 work. These included a laboratory program to evaluate the Purdue organisms and support pilot scale testing, a series of runs in the NREL process development unit (PDU), and process design and economic modeling.

Preparations began immediately, but the Amoco pretreatment reactor (APR), was not installed in the PDU until early spring of 1995. In addition, the cofermenting yeast were not yet available and Phase 3 work was initiated with the parent yeast strain 1400 which was capable of only glucose fermentation. The first PDU test on corn fiber, in February 1995, used the Sunds reactor for pretreatment. Two additional short runs were made in March and April, the first using the Sunds reactor and the second using the APR at NREL for the first time. After the third PDU run, work on Phase 3 was temporarily suspended because the original CRADA budget had been consumed.

During the summer, the Phase 3 scope of work was redefined (Appendix A-5). Acting on advice from commercial interests, the feedstock for Phase 3 was modified from corn fiber alone to a blend of corn fiber and corn screenings. Corn screenings are broken kernels of corn normally sold as an animal food. In the revised scope of work a series of seven project milestones, listed below, were established to guide the project to successful completion. All subsequent Phase 3 work directly supported one or more of these milestones.

1. Develop operating conditions for corn fiber/corn screenings blends in the APR.
2. Verify operability of all new or modified equipment in the PDU.
3. Determine if the Purdue recombinant yeast is suitable for corn fiber applications of SSCF technology.

4. Run the integrated PDU using the recombinant yeast on corn fiber at steady state, and in continuous mode to collect yield and performance data.
5. Develop process and economic models that successfully predict PDU behavior and use those models to select conditions that are commercially attractive for the production of representative solids in the PDU.
6. Produce enough representative solid product (10 tons) from corn fiber to conduct animal feeding tests
7. Produce a commercial design and cost estimate for the production of ethanol and animal feed from corn fiber that reflects the results from the Phase 3 program.

Laboratory Program and Kinetic Modeling

The purpose of the Phase 3 laboratory program and associated kinetic modeling was to develop an understanding of the performance of Purdue's cofermenting organisms in order to determine if they were suitable for corn fiber applications of SSCF technology (Milestone 3). Prior to the CRADA, these organisms were relatively untested in real world applications and little was known or understood regarding their performance in such situations. The SSCF kinetic model is also an essential part of the overall process model (Milestones 5 and 7).

Section 2 of this report summarized the results of the bench scale experiments conducted on three yeast strains provided by Purdue; Strain 1400, 1400(pLNH33) and 1400(LNHST2). The development of the SSCF kinetic model is also described.

1.2.2 PDU Operations

Most of the Phase 3 work involved running the PDU. The ultimate goals of these runs were to: 1) collect yield and performance data with the PDU operating in continuous mode using the Purdue recombinant yeast (Milestone 4), and 2) collect solid product (Milestone 6).

As part of the Phase 3 redefinition in the summer of 1995, five major tasks were identified for the PDU in order to support the Phase 3 milestones (these tasks came to be known as Tasks 1 through 5 respectively and are referred to as such throughout this report). They were:

1. Prepare the PDU for operation, including modification and installation of new equipment needed to meet the needs of the CRADA process.
2. Demonstrate PDU operability over the range of parameters of interest for the CRADA program.
3. Confirm the performance of the recombinant organism, LNHST2, at PDU scale in a series of batch runs.
4. Demonstrate continuous operation, with recycle of SSF broth and distillation.
5. Produce solid product for evaluation as an animal feed at optimum conditions based on a model of the process.

A total of seven runs were made in the PDU, the first four used Strain 1400 and SSF and the last three used the recombinant yeast, LNHST2, and SSCF. The runs with 1400 were useful for mechanical checking of plant operation and to demonstrate PDU operability over the range of parameters of interest for the CRADA program, but did not provide much relevant fermentation performance information. The last three PDU runs provided the best data and process performance. This information was used extensively in the conceptual process design and technoeconomic evaluation,

Solid residue from the process is an important coproduct due to its potential value as an animal feed supplement. Tests were carried out to determine the best method to separate the solids from the liquids in the

bottoms product from the distillation column. In addition, coproduct drying and pelletizing tests were carried out by equipment vendors. Finally, animal feeding tests were carried out on poultry, swine and cattle.

Section 3 includes summaries of PDU equipment operation, APR performance, fermentation performance, solid residue recovery experiments, animal feed test plans, predictions of the PDU kinetic model and overall PDU performance. Unfortunately, final results for the animal feeding tests are not available yet. They will be distributed as an addendum to this report when received from the subcontractors.

1.2.3 Technoeconomic Evaluation

A conceptual process design and technoeconomic evaluation was carried out to evaluate the commercial application of the CRADA biomass-to-ethanol technology to a feedstock blend of corn fiber and corn screenings (Milestones 5 and 7). It was assumed the feedstock would be provided by a corn wet mill with the biomass-ethanol unit installed in an integrated fashion with the mill.

Section 4 includes descriptions of the plant design basis, a process description, a description of the spreadsheet model developed to carry out the evaluation, a summary of the platform case economic evaluation and sensitivities to the platform case.

1.2.4. Recommendations

A review of the technical results presented in Sections 2, 3 and 4 lead to numerous conclusions regarding the status of the corn fiber to ethanol process developed under the CRADA and recommendations for how to improve the currently defined process. These recommendations are presented in Section 5.

1.3 Project Organization

The Phase 3 project team included numerous personnel from SWAN and NREL. Experimental plans were developed by designated team members and subject to approval by the CRADA Technical Steering Committee (TSC). Results were presented to the TSC and decisions were made regarding the relative success of the experiments and subsequent directions for future research. Members of the CRADA Phase 3 project team are:

SWAN
Robert. E. Lumpkin - TSC Chairman
Bob Lyons
Dixon Brandt - TSC Member
John Lesko - TSC Member
Lee Polite - TSC Member
Sam McWilliams
Larry Schwartz
Mark Grasso
Rick Houston
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Brian Boynton

Nancy Dowe
Jim Hora
Kelly Ibsen
Netta Ingle
Ed Jennings
Tim Johnston
George Philippidis
Tim Plummer
Fannie Eddy
Dana Rice
Mark Ruth
Susan Toon
Tina Ehrman

DOE
Gerson Santos-Leon ▪ TSC Member

2.0 Laboratory Program and Kinetic Modeling

Simultaneous saccharification and cofermentation (SSCF) is central to the conversion of corn fiber to ethanol. Recent development of cofermenting organisms, such as Purdue University's recombinant 1400(LNHST2), has enhanced the prospects for SSCF scale up and commercialization. However, the SSCF reaction pathway is complex and the organisms are new. Prior to the CRADA, LNHST2 was relatively untested in real world applications. The purpose of the Phase 3 laboratory program and associated kinetic modeling was to develop an understanding of the performance of Purdue's cofermenting organisms in order to determine if they were suitable for corn fiber applications of SSCF technology. This section summarizes the results of the bench scale experiments conducted on three yeast strains provided by Purdue and the development of the SSCF kinetic model.

Additional information on organism performance and kinetic modeling directly related to experiments in the PDU is included in Section 3.0.

2.1 Comparison of 1400, LNH33, and ST2

The key objective of the laboratory program was to develop bench scale data on organism performance before use in the PDU. Additionally, experiments also investigated inhibition, ethanol tolerance, and continuous performance. Three yeast strains described below were tested during the work.

Saccharomyces yeast strain 1400, a fusion product of *Saccharomyces diastaticus* strain 1384 and *Succharomyces uvarum* strain 21 (Stewart et al, 1982a), was used as a host strain by Purdue researchers (Ho et al, 1993; Ho and Tsao, 1995; and Ho and Chen, 1996) to incorporate xylose catabolism genes. Each fusion partner has unique characteristics that when fused together create a strain (1400) that contains the characteristics of both parents. Strain 1400 was chosen as the host stain due to its superior rate of glucose metabolism, ethanol and thermal tolerance over both fusion partners (Stewart et al, 1982b, Panchal, 1982).

Strain 1400(pLNH33), referred to as LNH33 in this report, was developed by transforming strain 1400 with a high-copy number plasmid containing three genes essential for xylose catabolism; xylose reductase (XR), xylitol dehydrogenase (XD), and xylulokinase (XK). LNH33, possessing the plasmid-borne ability to utilize xylose for ethanol production, was the first strain capable of cofermenting glucose and xylose tested under Phase 3 of the CRADA.

Since plasmid-borne traits tend to be unstable, a second strain capable of xylose catabolism was developed by Purdue researchers and tested at NREL. This strain, 1400(LNHST2), referred to as LNHST2 in this report, has the xylose catabolism genes (XR, XD, XK) incorporated into the chromosome of the host strain 1400 making the trait more stable than the plasmid version. Under Phase 3 of the CRADA, all three strains; 1400, LNH33, and LNHST2, were evaluated. However, only 1400 and LNHST2 were used in PDU runs.

2.1.1 *Saccharomyces sp.* 1400 glucose fermentation

Preliminary studies performed with 1400 investigated the effect of temperature, aeration and initial glucose level on the cell mass production and ethanol yield. This work identified process parameters for production of inoculum (seed train) and production of ethanol (fermentation train) in the PDU.

For production of inoculum, it is preferable to maximize the cell mass yield over ethanol production, which requires aeration and temperature control at 30°C. Increasing the initial level of glucose (from 20 g/L to 50 g/L) has a deleterious effect on the rate of cell mass production. However, a glucose level of 50 g/L does increase the final cell mass concentration.

For ethanol production the rate is increased by raising the temperature from 30°C to 37°C. The higher temperature also increases the rate of cellulose hydrolysis. Unfortunately, conditions that improve ethanol production are also optimal for glycerol production, a major by-product produced by 1400.

Ethanol Tolerance with 1400

A number of shake flasks experiments and a continuous fermentation were performed to examine the ethanol tolerance of 1400 (see Appendix A-l Laboratory Program, reports P4-P7). In a continuous fermentation on pure sugars, complete glucose utilization (50 g/L) was observed at a residence time of 12 hours in the presence of 60.5 g/L exogenous ethanol (see Appendix A-l Laboratory Program, report P4). The final concentration of ethanol was 66.2 g/L.

Simultaneous Saccharification and Fermentation (SSF)

Continuous and shake flask SSF were performed with 1400 and pretreated corn fiber, provided by Amoco in December of 1994 (see Appendix A-l Laboratory Program, reports P9-P10). These experiments demonstrated the ease of enzymatic digestion of cellulose in pretreated corn fiber. Shake flask experiments with an enzyme loading of 11.5 FPU cellulase/g cellulose showed 96.5% cellulose conversion in 7 days operating at 38°C and with a corn fiber slurry diluted to 40% w/w. A similar cellulose conversion (95.7%) was observed in a continuous SSF at a residence time of 56 hours operating at 34°C and the same solids level.

In both shake flask and continuous SSF experiments, the ethanol process yield based on total C6 sugars were low at 60.8% and 5 1.1% respectively, however the average metabolic yield was good at 77% of theoretical. The low process yields were due to unconverted C6 oligomers.

These SSF experiments demonstrated the ability of 1400 to ferment glucose present in corn fiber hydrolyzate. It was also shown that continuous inoculation was not necessary in a continuous SSF. However, future work focused on the co-fermentation strains, LNH33 and LNHST2.

2.1.2 Comparison of LNH33 and LNHST2 • Cofermentation

The growth rate of LNH33 on xylose alone was found to be 0.131 h“, substantially lower than on glucose (0.337 h^{-1}) (see Appendix A-l, Laboratory Program, report 1.3). In a batch fermentation with a mixture of the two sugars, a majority of glucose is consumed before xylose utilization begins. A two-stage (residence time of 24 hours per stage), continuous cofermentation with pure sugars, was conducted with glucose and xylose at levels representative of pretreated corn fiber. Complete glucose utilization occurred in the first stage, whereas only 26.3% conversion of xylose was observed at a 48-hour residence time (see Appendix A-l Laboratory Program, report 1 .5). The fermentation never reached a true steady state even after 11 residence times. This may be due to plasmid instability or a decrease in plasmid copy number leading to lower xylose utilization from one cell generation to the next.

A pure sugar, two-stage continuous cofermentation with LNHST2 was performed in the same manner as the previous experiment (see Appendix A-l Laboratory Program, report 1.6). A similar glucose utilization profile was observed, however, xylose utilization increased significantly. In the first stage, operating at a 23.3 hour residence time, 58.3% xylose conversion was observed, and 86.4% xylose conversion was observed at a 47-hour residence time (see Table 2.1 .1). LNHST2 appears to be more stable, since a steady state was quickly achieved. LNHST2 performed better, converting 92% of the sugars compared to 56% for LNH33. The growth rate of LNHST2 on glucose was 0.394 h^{-1} and 0.259 h^{-1} on xylose, slightly better than LNH33.

Table 2.1.1: Fermentation performance comparison of LNH33 and ST2 in a two-stage, pure sugar, continuous fermentation

Strain LNH33			
Stage	1	2	**Overall**
Residence Time (h)	24	24	48
Glucose Conversion:	100.0%		100.0%
Xylose Conversion	11.4%	16.9%	26.3%
Ethanol Process Yield (% theoretical)	40.1%	14.4%	47.6%
Ethanol Metabolic Yield (% theoretical)	84.3%	85.2%	84.4%
Strain LNHST2			
Stage	1	2	**Overall**
Residence Time (h)	23.3	23.3	46.6
Glucose Conversion:	99.5%	100.0%	100.0%
Xylose Conversion	58.3%	67.4%	86.4%
Ethanol Process Yield (% theoretical)	58.7%	47.2%	70.4%
Ethanol Metabolic Yield (% theoretical)	78.0%	69.7%	76.5%

2.1.3 LNHST2 Characteristics and Performance

The pure sugar studies showed that LNHST2 was a better cofermenter than LNH33. However, these studies were performed in the absence of metabolic inhibitors, such as acetic acid, lactic acid, HMF, furfural and lignin derived phenolics, present in pretreated material. Additional work examined the batch and continuous performance of LNHST2 with pretreated material.

During the course of bench scale testing with LNHST2, the composition of the pretreated corn fiber used for SSCF experiments changed significantly. Initial batch fermentations were performed with pretreated corn fiber containing only 3.9 g/L monomeric xylose and 2.0 g/L acetic acid at 20% total solids. Subsequent fermentations were performed with a mixture of pretreated corn fiber and corn screenings and that had been prepared using more severe pretreatment conditions. This new material contained significantly more monomeric xylose, 28 g/L at 20% total solids and slightly more acetic acid (2.5 g/L). A second batch of pretreated material contained the same initial monomeric sugar levels; however, the acetic acid concentration was almost double earlier values at 4.9 g/L (see Appendix A-l Laboratory Program, reports 1.7 and 1.8).

Table 2.1.2 summarizes the fermentation performance of LNHST2 with the mixture of pretreated corn fiber and corn screenings at the low (2.5 g/L) and high (4.9 g/L) acetic acid values. Xylose conversion was significantly lower at the higher acetic acid concentration (Batch 2). The maximum rate of glucose and xylose utilization was also lower in Batch 2.

Table 2.1.2: Summary of LNHST2 SSCF Performance at 20% solids level

	Batch 1* 20% solids low acetic acid	Batch 2* 20% solids high acetic acid
Total Glucose Conversion (%):	74.1	78.4
Monomeric Xylose Conversion (%):	92.8	68.1
Total Xylose Conversion (%):	62.8	56.1
Maximum Monomeric Glucose Utilization Rate (g/L-h)	3.52	2.41
Maximum Monomeric Xylose Utilization Rate (g/L-h)	0.91	0.25
Ethanol Process Yield (% theoretical):	56.9	63.5
Ethanol Metabolic Yield (% theoretical)	80.5	88.1

* The duration of each run was 113 h for batch 1 and 167 h for batch 2

The inhibitory effect of acetic acid on xylose utilization and ethanol production has been confirmed by studies performed with a variety of yeast strains at various pH levels (Zyl et al., 1991; Ramos and Madiera-Lopes, 1990). The incomplete use of monomeric xylose is probably due to acetic acid inhibition (and perhaps other inhibitors).

This research prompted further experimentation examining the effect of acetic acid, HMF and furfural on the fermentation performance of LNHST2. An experiment performed by Purdue researchers (see Appendix A-l laboratory Program, report 1.9) showed that the presence of organic acids inhibits sugar utilization, with acetic acid being more inhibitory than lactic acid. It should be noted that the pH in this experiment was not controlled, and therefore, the contribution of pH to the inhibition of sugar utilization cannot be determined. When the pH is lower than the pk, of acetic acid, the inhibitory effect of the acid becomes significantly stronger. The Purdue data showed that xylose fermentation is vulnerable to the simultaneous presence of ethanol and acids. Even low acid concentrations (5 g/L total lactic and acetic acids), typical of pretreated material, inhibited xylose uptake at 30 g/L ethanol.

In addition to the detrimental effect of acetic acid on xylose uptake and ethanol production, the presence of furfural seems to correlate with the lag phase observed during SSCF with LNHST2. Once the furfural concentration drops below 0.2 g/L, exponential growth begins. It has been reported in the literature that a lag phase is observed and cell death occurs in the presence of HMF and furfural, and cell growth begins with elimination of these compounds from the culture (Chung and Lee, 1985). In our studies, HMF utilization occurs during both the lag and exponential phases and it reaches near zero as glucose approaches zero. (See Appendix A-2.5, Task S Run Report.)

Other studies with LNHST2 have shown that xylose conversion is better when sodium hydroxide (NaOH) is used for pH adjustment and control when compared to ammonium hydroxide (NH_4OH). In a pure-sugar shake flask study, 65% xylose conversion was observed when NH_4OH was used for pH adjustment, compared with 84% conversion with NaOH after 91 hours of fermentation.

All of the experiments performed with the recombinant yeast strains were performed at 30°C due to poor xylose utilization at 37°C.

2.1.4 Conclusion

Although strain 1400 cannot ferment xylose, its fermentation characteristics made it a good candidate for genetic engineering. Therefore, work conducted at Purdue added xylose fermenting genes to 1400. Two transformants created with 1400, LNH33 and LNHST2 were tested at NREL under the CRADA. Continuous and batch studies have demonstrated that the chromosome-integrated genes of LNHST2 enable the organism to metabolize xylose in addition to glucose more effectively than the plasmid bearing strain, LNH33.

Acetic acid and lactic acid have been shown to inhibit xylose utilization. High concentrations of furfural and HMF may play a role in the duration of the lag phase observed in SSCF as well as affect the rate of glucose utilization. Data generated from the experiments performed at the bench scale with LNHST2 were used to develop the kinetic model described in Section 2.2.

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2.2 SSCF Kinetic Model

2.2.1 Introduction

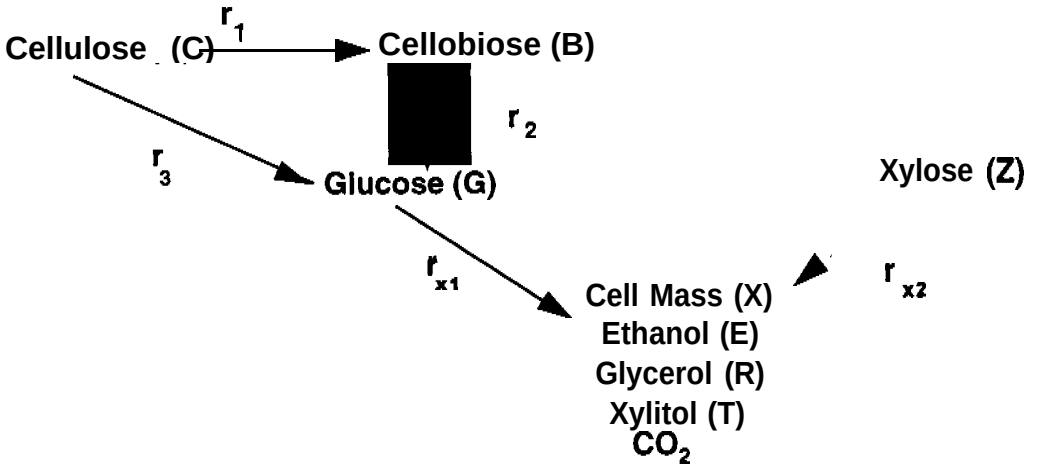
A simple kinetic model of SSCF with pretreated corn fiber and screenings was developed to assist with optimization. The model can be useful in finding parameter values (e.g., residence time, enzyme loading, solids concentration) that minimize the ethanol production cost when linked with the techno-economic model. The kinetic model describes the rates of the cellulose saccharification and glucose and xylose fermentation reactions as functions of saturation terms, inhibition terms, and catalyst concentrations.

The SSCF reaction pathway is complex, combining the heterogeneous nature of cellulose hydrolysis to cellobiose and glucose, the hydrolysis of soluble cellobiose to glucose, and the fermentative production of ethanol from both glucose and xylose'. Furthermore, the rate of the SSCF process is affected by the potential inhibitory effect of:

1. Feedstock components and pretreatment products, such as acetic acid, lactic acid, furfural, hydroxymethyl furfural (HMF), and lignin-derived phenolics, on cell metabolism;[5]
2. Hydrolysis products, such as cellobiose and glucose, on enzyme activity, primarily through end product inhibition;[6]
3. Metabolic products, such as ethanol, on enzymatic and cellular activities;[6] and
4. By-products from low-level microbiological contaminants, such as acetic acid and lactic acid.

Hence, a successful SSCF model needs to encompass the structural characteristics of the cellulosic substrate, the specific activity and properties of the cellulase enzyme complex, the interaction between substrate and enzyme, and the interaction between substrate and femmentative organism. In addition, the model's simplicity should be preserved to enhance its practical usefulness.

NREL's previously described SSF model',' was expanded to incorporate xylose fermentation kinetics. The SSCF model consists of two interdependent parts. The first describes the enzymatic hydrolysis kinetics for cellulose and depends on the characteristics of the particular enzyme-substrate system. The second describes the fermentation kinetics for glucose and xylose and depends on the characteristics of the fermentative organism. In the present version of the model, the fermentation kinetics are formulated specifically for the recombinant yeast strains, which ferment glucose and xylose to ethanol with cell mass, CO_2, glycerol, and xylitol being the main by-products, according to the following scheme:



2.2.2 Hydrolysis Parameter Determination

Based on previous experimental data', the rates for cellulose and cellobiose hydrolysis are given by the following expressions for cellulose hydrolysis to cellobiose;

$$r_1 = \frac{k_1' C e^{-\lambda(1-C/C_o)}}{1+\frac{B}{K_{1B}}+\frac{G}{K_{1G}}} \frac{K_{1E}}{K_{1E}+E} \qquad (1)$$

cellulose hydrolysis to glucose;

$$r_3 = \frac{k_3' C e^{-\lambda(1-C/C_o)}}{1+\frac{B}{K_{1B}}+\frac{G}{K_{1G}}} \frac{K_{1E}}{K_{1E}+E} \qquad (2)$$

and cellobiose hydrolysis to glucose;

$$r_2 = \frac{k_2' B}{K_m(1+\frac{G}{K_{2G}}) + B} \tag{3}$$

where C, B, G, and E are the concentrations (g/L) of cellulose, cellobiose, glucose, and ethanol, respectively, C_0 is the initial cellulose concentration (g/L), K_{1B}, K_{1G}, K_{1E}, and K_{2G} are inhibition constants (g/L), as detailed in the "Nomenclature" section, k_1', k_2', and k_3' are the lumped specific rate constants for cellulose (h^{-1}) and cellobiose (g/L-h) hydrolysis, and λ is the rate of decrease in the specific surface area of cellulose during the course of the enzymatic hydrolysis.

It should be noted that unlike the SSF model', here we consider the gradual loss in surface area (or enzyme mobility') a function of cellulose conversion (C/C_0) rather than time. This way, the progress of the reaction becomes directly dependent on the extent of cellulose hydrolysis, an intrinsic parameter of both the SSF and SSCF systems.

The lumped specific rate constants of the heterogeneous cellulose hydrolysis, k_1', and k_3', exhibit a Michaelis-Menten dependence on the cellulase concentration according to the following expression';

$$k_i' = \frac{k_i^*(e)_T e_c^*}{K_{eq} + (e)_T e_c^*} \quad , \qquad i = 1,3 \tag{4}$$

where $(e)_T$ is the total (free and bound) concentration of the cellulase and β-glucosidase enzyme complex (g/L), e_C^* is the specific cellulase activity of the enzyme preparation (IFPU/g protein), k_i^* are the maximum specific cellulose hydrolysis rates (h^{-1}), and K_{eq} is the cellulase enzyme saturation constant (g/L). On the other hand, the lumped specific rate constant k_2' of the homogeneous cellobiose hydrolysis is proportional to the concentration of β-glucosidase, taking into account the loss of enzyme activity through irreversible adsorption to the lignin present in the biomass';

$$k_2' = k_2^*(e)_T e_g^* [1 - K_L(L)] \tag{5}$$

where k_2^* is the specific cellobiose hydrolysis rate (g/IU-h), e_g^* is the specific β-glucosidase activity of the enzyme preparation (IFPU/g protein). L is the lignin concentration (g/L), and K_L is the β-glucosidase adsorption to lignm constant (L/g).

A series of experiments were conducted to determine the rates of cellulose hydrolysis and fermentation for APR-treated corn fiber. A multivariate, nonlinear, least-squares parameter-fitting procedure using the software package Scientist (MicroMath, Salt Lake City, UT) was employed to solve the algebraic-differential system of the model equations and determine the best values of the adjustable parameters; k_1', k_2', k_3' and lambda (h).

The data were generated from an enzymatic saccharification experiment (Technical Record Book #1749 pp. 19-31) with 20% (w/w) corn fiber and screenings pretreated with sulfuric acid in the APR.

Six enzyme dosages were studied (5, 10, 15, 18, 36, and 54 IFPU/g cellulose). The solids concentration (20%) and operating conditions (30°C, pH 5.0, 150 rpm) in these experiments were selected to reflect realistic SSCF conditions. Samples were taken every 30 minutes during the first hour, hourly during the following 5 hours, and less frequently afterwards. The samples were analyzed through HPLC for cellobiose and glucose.

The time courses of released glucose and cellobiose (first 48 hours) were used to fit the model equations by adjusting the three specific rate constants, k_1', k_2', k_3' and λ of Equations (1)-(3). The values of the remaining parameters of the enzymatic hydrolysis expressions (inhibition constants for cellulase and β-glucosidase) were taken from a previous NREL study", since the cellulolytic enzymes used in the CRADA work are assumed to be comparable with those employed in previous work. As seen in Figures 1 and 2 (5 and 15 IFPU/g, respectively), the model equations fit the experimental data in a satisfactory way. Similarly good agreements between model predictions and data were obtained at all the examined enzyme dosage levels. The derived optimal parameter values from this series of enzymatic hydrolysis experiments are summarized in Table 2.2.1.

Table 2.2.1. Optimal parameter values for the hydrolysis of corn fiber by cellulase.

	Enzyme Loading (IFPU/g cellulose)					
Parameter	**5**	**10**	**15**	**18**	**36**	**54**
k_1' (h^{-1})	0.011532	0.020944	0.028668	0.043481	0.12046	0.19327
k_2' (g/L h)	1.4387	3.3413	5.5157	7.1534	14.866	19.262
k_3' (h^{-1})	0.0028426	0.0026878	0.0033026	0	0	0.010503
λ	2.7741	0.53749	0	0.1239	1.0173	1.5205

Figure 2.2.1. Experimental data and mode1 predictions for the enzymatic hydrolysis of pretreated corn fiber in the presence of 5 IFPU/g cellulose (C: cellulose, B: cellobiose, G: glucose).

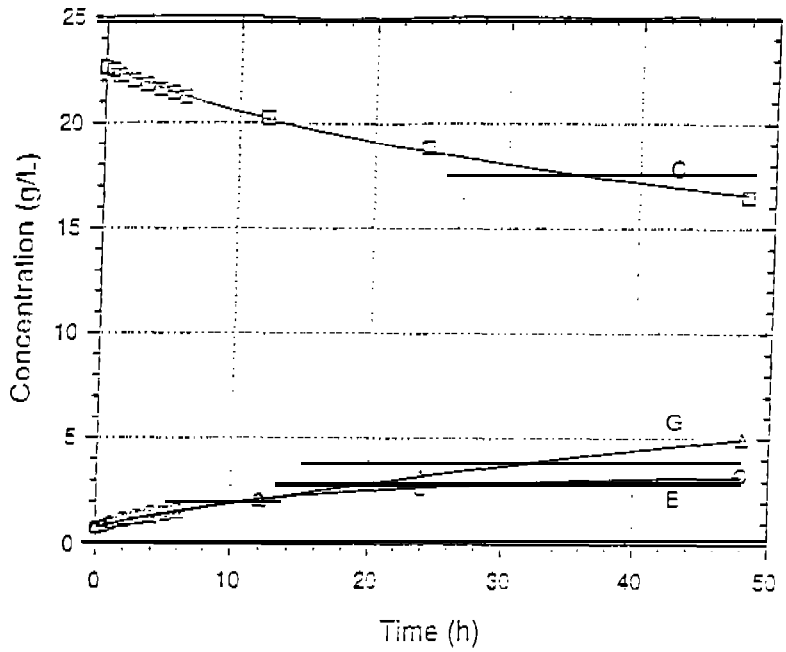
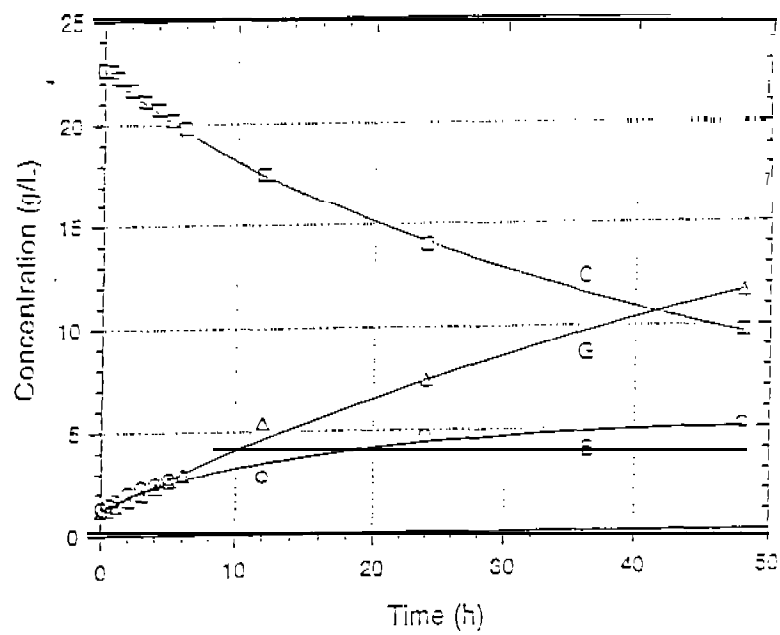


Figure 2.2.2. Experimental data and model predictions for the enzymatic hydrolysis of pretreated corn fiber in the presence of 15 IFPU/g cellulose (C: cellulose, B: cellobiose, G: glucose).



Increasing the enzyme dosage 10.8 fold (5 IFPU to 54 IFPU/g cellulose) enhanced the k_1' value by 16.8 fold. This increase far exceeded the expected increase expressed in Equation (4). It is assumed that the loadings are far below the saturation level, so the saturation term was dropped. The expression for cellulose hydrolysis to cellobiose follows;

$$k_1' = k_1^* (e),\ e_c^* \tag{6}$$

The same 10.8 fold increase in the enzyme dosage enhanced the k_2' value by 13.4 fold. The specific rate for cellobiose to glucose was expressed as proportional to enzyme loading, so there was no saturation term to drop. However the lignin adsorption term was dropped, because corn fiber/screenings has a low lignin content. The modified expression for cellobiose hydrolysis to glucose follows;

$$k_2' = k_2^* (e),\ e_g^* \tag{7}$$

Moreover, the specific rate of cellulose hydrolysis to glucose, k_3', did not follow an increasing trend and varied between zero and 0.010503 h^{-1} with a mean value of 0.00322 h^{-1}. Therefore, k_3' was modeled as a constant.

Similarly, the value of λ varies between zero and 2.7741 with no apparent trend. This is a result of our limited understanding of the phenomena involved in the loss of enzyme mobility and/or substrate reactivity during the course of enzymatic hydrolysis, which is the rate-limiting step of SSF and, hence, of SSCF.

The discrepancies are believed to be a consequence of the model's simplicity; the model omits the formation of intermediary oligomers from cellulose, because they cannot be measured experimentally, and assumes that cellobiose and glucose are formed directly from cellulose. Hence, the more-than-proportional increase in k_1' and k_2' may explain the very low values of k_3'.

The cellulose hydrolysis parameters developed from this experiment and used throughout Phase 3 are shown in Table 2.2.2.

Table 2.2.2. Parameter values for the hydrolysis of corn fiber by cellulase used throughout Phase 3.

Parameters	Values
k_1^* (IJIFPU-h)	0.0000201
e_c^* (IFPU/g enz)	410
λ	1.104
K_{IB} (g/L)	5.85
K_{IG} (g/L)	53.16
K_{IE} (g/L)	50.35
k_2^* (L/IU-h)	0.00356
e_e^* (IU/g enz)	820
K_M (g/L)	10.56
K_{2G} (g/L)	0.62
k_3' (h^{-1})	0.002944

2.2.3 Fermentation Parameter Determination

2.2.3.x Batch SSCF Model

The fermentation part of SSCF was initially modeled with the following expressions describing the rate of glucose utilization as;

$$r_{X1} = \mu_{m1} X \frac{G}{K_G+G} \frac{K_E}{K_E+E} \tag{8}$$

and xylose utilization as;

$$r_{x2} = \mu_{m2} X \frac{Z}{K_Z+Z} \frac{K_E}{K_E+E} \frac{1}{1+G/n} \tag{9}$$

where X and Z are cell mass and xylose concentrations (g/L). respectively, μ_{m1} and μ_{m2} are maximum specific growth rates (h^{-1}) of the yeast on glucose and xylose, respectively, K_G and K_Z are glucose and xylose saturation constants (g/L), respectively, and K_E is the product (ethanol) inhibition constant (g/L). The parameter n is a

factor that accounts for the experimentally documented preferential uptake of glucose over xylose (diauxic phenomenon). Better cofermentation performance is associated with a larger n.

Based on the outlined rate expressions, the following mass balance equations describe the batch SSCF process for cellulose concentration;

$$\frac{dC}{dt} = -r_1 - r_3 \tag{10}$$

cellobiose concentration;

$$\frac{dB}{dt} = 1.056r_1 - r_2 \tag{11}$$

glucose concentration;

$$\frac{dG}{dt} = 1.111r_3 + 1.053r_2 - \frac{r_{X1}}{Y_{XG}} \tag{12}$$

xylose concentration;

$$\frac{dZ}{dt} = -\frac{r_{X2}}{Y_{XZ}} \tag{13}$$

cell mass concentration;

$$\frac{dx}{dt} = r_{X1} + r_{X2} \tag{14}$$

glycerol concentration;

$$\frac{dR}{dt} = r_{X1}\frac{Y_{RG}}{Y_{XG}} + r_{X2}\,Y_{XZ} \tag{15}$$

xylitol concentration;

$$\frac{dT}{dt} = r_{X2} \frac{Y_{TZ}}{Y_{XZ}} \quad (16)$$

and ethanol concentration;

$$E = E_o - 1.278[0.444(C-C_o) + 0.4(G-G_o) + 0.4(Z-Z_0) + 0.421(B-B_o) + 0.391(R-R_o) + 0.394(T-T_o) + 0.479(X-X_0)] \quad (17)$$

where Y_{XG} and Y_{XZ} are cell mass yields from glucose and xylose (g/g), respectively, Y_{RG} and Y_{RZ} are glycerol yields (g/g) from glucose and xylose, respectively, and Y_{TZ} is xylitol yield (g/g) from xylose. Cell mass is formed from the consumption of both glucose and xylose, and glycerol is a by-product generated during the catabolism of both sugars. In contrast, xylitol formation takes place only as a result of inefficient xylose metabolism. In equations (11) and (12), the numeric constants account for the mass gain per mole of reactant caused by hydration during the hydrolysis reactions (if concentrations are expressed in moles, all constants should be set equal to one). It should be noted that the mass balance expression for ethanol, equation (17), ensures carbon balance closure for the fermentation and is based on carbon and degree of reduction balance considerations'.

2.2.3.2 Kinetic Parameters for LNH33 and LNHST2

The initial growth and product formation parameters of the SSCF model were determined by cultivating LNH33 and LNHST2 on mixtures of pure glucose and xylose. During the course of the fermentation, the concentration profiles of the two sugars, ethanol, cell mass, glycerol, and xylitol were monitored and subsequently fitted to the fermentation expressions, Equations (12)-(17). It should be noted that the glucose expression, Equation (12), was modified to include only the consumption term, since there was no glucose formation from cellulose and cellobiose in the pure sugar experiments. The fermentation conditions were: 30°C, pH 5.0, 150 rpm, and 1% CSL. The potential inhibitory effects of organic acids, phenolics, and other hydrolyzate constituents on cell metabolism were not taken into account.

As Figures 2.2.3 and 2.2.4 show, the model equations provide a good lit to the experimental data for both organisms. LNH33 is unable to coferment the two sugars, instead, it starts utilizing xylose only after glucose has been exhausted. In contrast, LNHST2 co-metabolizes the two sugars, but consumes glucose at a significantly higher rate than xylose. The determined values of the parameters for each organism are summarized in Table 2.2.3.

Figure 2.23 Experimental data and model predictions for the cell (X) growth and ethanol (E) production by LNH33 cultivated in a mixture of glucose (G) and xylose (Z) in batch mode (R: glycerol, T: xylitol).

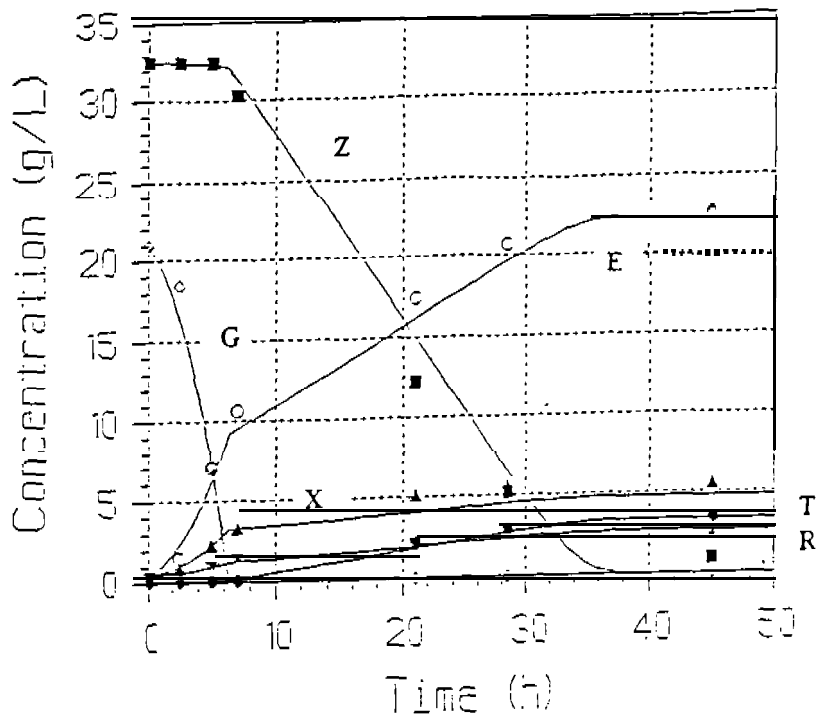


Figure 2.2.4 Experimental data and model predictions for the cell (X) growth and ethanol (E) production by LNHST2 cultivated in a mixture of glucose (G) and xylose (Z) in batch mode (R: glycerol, T: xylitol).

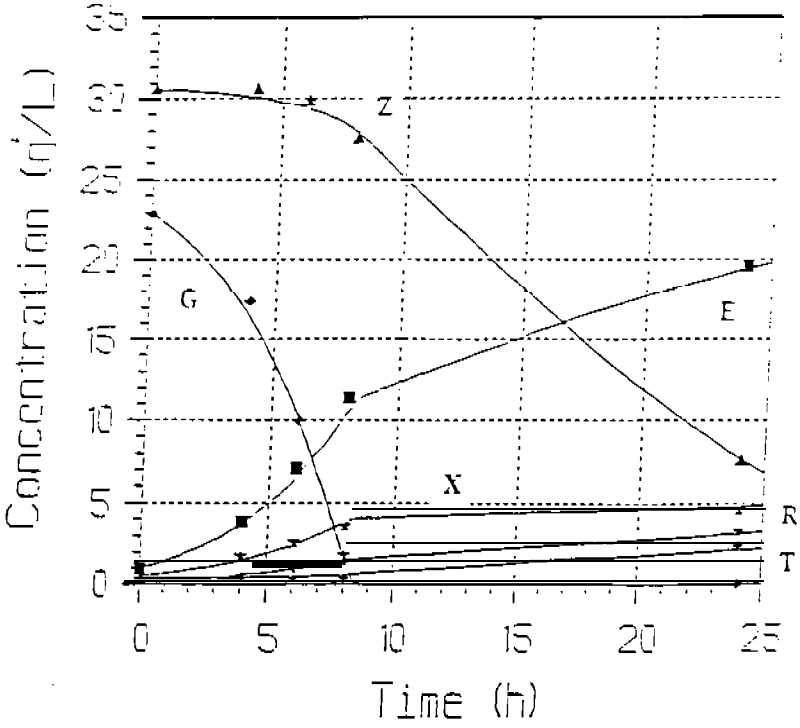


Table 2.2.3. Optimal parameter values for LNH33 and LNHST2 cultivated on a mixture of glucose and xylose.

Kinetic Parameters[a]	LNH33[b]	LNHST2[c]
$\mu_{m1}(h^{-1})$	0.353	0.292
K_E(g/L)	27.81	29.29
K_G(g/L)	0.148	0.385
$\mu_{m2}(h^{-1})$	0.024	0.024
K_X(g/L)	1.13	7.25
Y_{XG}(g/g)	0.131	0.150
Y_{XZ}(g/g)	0.048	0.044
Y_{RG}(g/g)	0.033	0.082
Y_{RZ}(g/g)	0.043	0.038
Y_{TZ}(g/g)	0.099	0.077
n	1.00	8.75

"The parameters are listed in the nomenclature at the end of this report.

[b] Cultivated in 1% yeast extract, 2% peptone, 21 g/L glucose, and 32.5 g/L xylose in batch mode for 45 hours (see Report 1.3, flask #2).

'Cultivated in 1% yeast extract, 2% peptone, 22.86 g/L glucose. and 30.61 g/L xylose in batch mode for 24 hours before switching the operation to continuous (see Report 1.6).

The parameter values reveal strong similarities between the two strains, as expected, since they are both descendants of L1400. LNH33 grows slightly faster on glucose than LNHST2 (a doubling time of 1.96 hours versus 2.37 hours), but they both grow at the same rate on xylose. Interestingly, the doubling time on xylose is 28.9 hours, about 14-fold slower than on glucose for both strains. The two organisms have similar ethanol tolerance with a mean K_E value of about 28.6 g/L. Thus, the growth rate at 28.6 g/L ethanol is half of that in the absence of ethanol inhibition. With regard to sugar preference, the K_G values are 7.6-fold (LNH33) and 18.8-fold (LNHST2) smaller than the respective K_Z values, confirming the experimental observations that glucose is preferred to xylose by the cells (see Appendix A-l, Report 1.4).

With respect to the yield coefficients, there are some differences between the two organisms. LNHST2 makes 14.5% more cell mass (Y_{XG}) from glucose (0.150 g/g) than LNH33 (0.131 g/g) and 1.5-fold more glycerol (Y_{RG}) from glucose (0.082 versus 0.033 g/L). On the other hand, LNHST2 makes 8.3% less cell mass from xylose (Y_{XZ}), 11.6% less glycerol from xylose (Y_{RZ}), and 22.2% less xylitol (Y_{TZ}).

The most significant advantage of LNHST2 over LNH33 is its ability to coferment glucose and xylose (n=8.75 for LNHST2 versus n=l for LNH33). The implications of this difference are especially significant for continuous operations, where the simultaneous presence of both sugars (at least in the first stages of the SSCF tram) will result in suppression of xylose utilization in LNH33. No further work was done on LNH33 because only LNHST2 was designated for use in the PDU.

After the pure sugar experiments, the ethanol inhibition term (K_E) was split to better describe ethanol's inhibitory effects on xylose utilization. Two new terms, $K_{E,G}$ and $K_{E,Z}$, were added to express ethanol inhibition on the glucose and xylose pathways, respectively. The terms' values were determined from bench scale experiments (Appendix A-l, Report 1.6).

Cell mass yields (Y_{XG} and Y_{XZ}) were set to 0.05 g/g. Cell mass yields in the shake flasks were higher than 0.05 g/g, but the yields in the chemostat and the PDU were near 0.05 g/g. Two possible causes of the discrepancy are increased oxygen transfer in the shake flasks and higher glucose concentrations at the beginning of batch fermentations when compared to continuous fermentations. The constants μ_{m1}, μ_{m2}, K_Z, and n were modified from the values shown in Table 2.2.3 so that the model's predictions would better fit Task 3 data. The values of μ_{m1}, μ_{m2}, K_Z, and n after Task 3 were 0.13 g/L h, 0.08 g/L h, 250 g/L, and 50 g/L, respectively.

2.2.3.3 Organic Acid Inhibition of Xylose Utilization

A shake flask study was conducted to study the effects of dilution on fermentation performance of two hydrolysates (APR-330 and APR-392) (see Appendix A-2, Task 5 Run Report, Appendix C). Shake flask fermentations of the liquor from each hydrolysate were performed at the equivalent of 25%, 18%, and 12% solids. Shake flask fermentations of the whole hydrolysate from APR-392 were performed at 25% and 12% solids.

The xylose utilization rate was found to be dependent upon the sum of the concentrations of acetic and lactic acid, so a term was added to the xylose utilization expression. The modified xylose utilization expression is;

$$r_{x2} = \mu_{m2} X \frac{Z}{K_Z + Z} \frac{K_{E,Z}}{K_{E,Z} + E} \frac{1}{1 + G/n} e^{-K_{Z,TA}A} \qquad (18)$$

where $K_{Z,TA}$ is the acid inhibition constant and A is the sum of the acetic acid and lactic acid concentrations (g/L). The exponential expression was chosen because it fit the data better than a Monod term or a straight line with a Y intercept equal to 1. A straight line intercept of 1 is necessary because the expression must equal one when no organic acids are present.

The maximum utilization rate of xylose (μ_{m2}), the xylose saturation term (K_Z), and the cofermentation constant (n) were modified to fit the new model to both shake flask experimental data (Task 5 Run Report, Appendix C) and Task 3 data. When Task 3 data was initially fit, the xylose saturation constant was set to 250 g/L to account for the increased xylose utilization rate soon after glucose disappears. However, the xylose saturation constant was determined to be 15 g/L from the organic acid inhibition experiment data, so increased xylose utilization soon after glucose disappears needs to be accounted for in another way. The cofermentation constant was increased to 1000 g/L because glucose concentration seems to have little impact on xylose utilization. The slow xylose utilization rate at the beginning of batch fermentations is probably caused by lower cell mass concentrations. When glucose has disappeared the resulting increase in cell mass concentration increases the xylose utilization rate. The measured and modeled glucose, xylose, and ethanol concentrations and the modeled cell mass concentration for the 8 shake flasks and Task 3 are shown in Appendix A-2 (Task 5 run report, Appendix F). The modeled fermentation start time was estimated to fit the glucose data in each flask, because there was not enough information to properly model inhibition.

Xylose concentration at each data point was found to be close to the model's prediction. The measured and predicted xylose concentrations are close when glucose is present, the measured value is lower for approximately 30 hours after glucose is gone, and near the end of the fermentation the values are close again. Apparently, another factor needs to be added to the model to account for the increased xylose utilization rate for a period of time after glucose disappearance.

2.2.3.4. HMF Inhibition of Glucose Utilization

In the literature, it has been noted that furfural can cause inhibition at the start of batch ethanol fermentations until it has been metabolized[9]. An experiment was conducted to further investigate the possible inhibitory effects of both HMF and furfural (see Appendix A-2, Task 5 Run Report, Appendix D). The experiment consisted of 3 shake flask fermentations of clarified hydrolyzate produced by the APR on June 14, 1996 (between APR-417 and APR-418) at 25%, 18%, and 12% equivalent solids.

The data showed disappearance of both HMF and furfural. Following are the kinetic expressions that were developed to model the disappearance of furfural;

$$r_U = k_U' X \frac{U}{K_{U,U}+U} \tag{19}$$

and the disappearance of HMF;

$$r_H = k_H' X \frac{H}{K_{H,H}+H} \tag{20}$$

where r_U is the rate of futfural conversion (g/L-h), k_U' is the maximum furfural conversion rate (h^{-1}), X is the cell mass concentration (g/L), U is furfural concentration (g/L), $K_{U,U}$ is the furfural saturation constant (g/L), r_H is the rate of HMF conversion (g/T--h), k_H' is the maximum HMF conversion rate (g/L-h), H is the HMF concentration (g/L), and $K_{H,H}$ is the HMF saturation constant (g/L).

The glucose inhibition seems to be more dependent upon HMF than on furfural (see Appendix A-2, Task 5 Run Report, Appendix F). Therefore, a Monod kinetic term was added to the glucose utilization rate expression to account for HMF inhibition. The updated glucose utilization rate equation follows;

$$\mu_{ml} X \frac{G}{K_G+G} \frac{K_{E,G}}{K_{E.G}+E} \frac{K}{K_G} \tag{21}$$

where $K_{G.H}$ is the HMF inhibition constant (g/L). The corrected glucose utilization term was further corrected by dividing out the HMF inhibition term. No correlation appears to be present between furfural concentration and further inhibition. Furfural may be a cause of inhibition, but the inhibition seen in this data was better expressed by the HMF inhibition term. More experimental work is necessary to separate the effects of HMF and furfural and to correctly model the effects of HMF or furfural concentrations greater than 0.4 g/L.

Most likely, HMF and/or furfural also inhibit xylose utilization. However, the predicted xylose consumption while HMF and furfural was present in the bench scale experiment (Appendix A-2, Task 5 Run Report, Appendix D) was minimal, so any change in xylose consumption was not detected.

The fermentation performance of each of the three shake flasks was then modeled with the updated glucose utilization rate equation and the maximum glucose utilization rate (μ_{ml}) was modified to fit the HMF inhibition term. The final fermentation terms determined after these experiments are shown in Table 2.2.4.

Table 2.2.4. Kinetic Parameters after the Hydrolysate Dilution Experiment and after the HMF Inhibition Experiment.

Kinetic Parameters[a]	Final Parameters
$\mu_{m1}(h^{-1})$	***0.22***
$K_{E,G}$(g/L)	***73.7***
K_G(g/L)	***0.385***
$K_{G,H}$(g/L)	0.3
$\mu_{m2}(h^{-1})$	0.06
K_Z(g/L)	15
$K_{E,Z}$(g/L)	***21***
(L/g)	***0.25***
Y_{XG}(g/g)	***0.05***
Y_{XZ}(g/g)	0.05
Y_{RG}(g/g)	0.082
Y_{RZ}(g/g)	0.038
Y_{IZ}(g/g)	0.077
n (g/L)	1000
k_U' (h^{-1})	0.08
$K_{U,U}$ (g/L)	0.04
k_H' (h^{-1})	0.05
$K_{H,H}$ (g/L)	0.1

'Parameters are defined in the nomenclature at the end of this section.

2.2.3.5 Continuous Fermentation

Predictions from the continuous model were compared to steady state conditions in the PDU during Task 5 and chemostat runs. The batch expressions (eq. 10—16) were integrated to develop the continuous model, under the assumption that all of the fermenters are CSTRs.

At steady state conditions, the continuous kinetic model over-predicted xylose utilization. The over-prediction seemed to be most prevalent in the first fermenter, so a term was added to reduce the predicted cell mass concentration in the first fermenter. Before this change, the cell mass concentration in all of the fermenters was modeled with the following expression;

$$\frac{F_{i-1}X_{i-1}}{V} - \frac{F_iX_i}{V} + (r_{x1} + r_{x2}) = 0 \qquad (22)$$

where F_{i-1} is the volumetric flow rate (L/h) entering the fermenter, X_{i-1} is cell mass concentration entering the fermenter (g/L), V is the fermenter volume (L), F_i is the volumetric flow rate leaving the fermenter (L/h), X_i is the cell mass concentration in the fermenter (g/L), and r_{x1} and r_{x2} are cell mass production rates (g/L-h) as described in equations 21 and 18, respectively. The equation for the first fermenter was changed to the following;

$$\frac{X_{i-1}}{/} - \frac{F_i X_i}{v} + (r_{x1} + r_{x2} - r_x \tag{23}$$

where r_{x3} is the cell mass reduction term. The cell mass reduction term is described by the following expression;

$$\% \quad \mathbf{Z} - \frac{F_i}{V} \mathbf{i} \, r'_{x3} \tag{24}$$

where r_{x3}' is the cell mass reduction term without a time unit (g/L). This term (r_{x3}') accounts for a reduction in cell mass yield while metabolizing furfural and/or HMF. However, the reason for the cell mass yield reduction is unknown so the use of this term should be studied further. Since all of the furfural and HMF disappear from the first fermenter at residence times of 24-36 hours used in the chemostat and PDU, the reduction was made independent of time.

The cell mass reduction term (r_{x3}') was found for each of the four steady states achieved in the chemostat and PDU during Task 5, by forcing the predicted xylose concentration in the last fermenter (the second in the chemostat and the third in the PDU) to be equal to the measured xylose concentration. Cell mass reduction appears to be dependent upon both furfural and HMF levels. Further experiments are needed to separate the effects of HMF from those of furfural and to investigate if cell mass yields are reduced in continuous fermentation when little or no HMF or furfural is present.

Cell mass reduction is expressed as a function of furfural in the following equation;

$$r'_{x3} = 5.27 U_0 - 1.07 \tag{25}$$

where U_0 is furfural concentration in the feed to SSCF (g/L).

Chemostat runs #3 and #4 (Appendix A-2: Task 5 run report, Appendix E) were modeled using the final equations and parameters, and the model results were compared to measured data. The model predicted minimal glucose in the first fermenter, but a large concentration was measured in it. The cause of the large concentration is unknown. This unmetabolized glucose may be caused by metabolism of furfural and HMF that is not accounted for by the cell mass removal term. If unconverted glucose in the first fermenter were converted, the ethanol concentration would be close to the predicted value. The xylose concentration in the first fermenter is lower than predicted at both steady states. This lower concentration may be related to a rapid utilization of xylose after glucose disappears that is not accounted for by the kinetic model.

Nomenclature

A	Sum of the Concentrations of Acetic Acid and Lactic Acid (g/L)
B	Concentration of cellobiose (g/L)
C	Concentration of cellulose (g/L)
C_0	Concentration of cellulose before hydrolysis (g/L)
$(e)_T$	Concentration of cellulase and β-glucosidase enzyme complex (g protein /L)
e_c^*	Specific cellulase activity of the enzyme preparation (IFPU/g protein)
e_g^*	Specific β-glucosidase activity of the enzyme preparation (IU/g protein)
E	Concentration of ethanol (g/L)
F_{i-1}	Volumetric flowrate entering the fermenter (L/h)
F_i	Volumetric flowrate leaving the fermenter (L/h)
G	Concentration of glucose (g/L)
H	Concentration of HMF (g/L)
k_1^*	Maximum specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2^*	Maximum specific rate of cellobiose hydrolysis to glucose (g/IU-h)
k_3^*	Maximum specific rate of cellulose hydrolysis to glucose (g/IU-h)
k_1'	Lumped specific rate of cellulose hydrolysis to cellobiose (h^{-1})
k_2'	Lumped specific rate of cellobiose hydrolysis to glucose (g/L-h)
k_3'	Specific rate of cellulose hydrolysis to glucose (h^{-1})
K_E	Averaged ethanol inhibition constant in the microorganism (g/L)
$K_{E,G}$	Ethanol inhibition constant for glucose pathway in the microorganism (g/L)
$K_{E,Z}$	Ethanol inhibition constant for xylose pathway in the microorganism (g/L)
K_{eq}	Cellulase enzyme saturation constant (g/L)
K_G	Glucose saturation constant for the microorganism (g/L)
k_H'	Maximum HMF conversion rate (g/L-h)
$K_{G,H}$	HMF inhibition of glucose utilization constant (g/L)
$K_{H,H}$	HMF saturation constant (g/L)
K_L	β-glucosidase adsorption to lignin term (L/g)
K_m	Cellobiose saturation constant for β-glucosidase (g/L)
K_{IB}	Inhibition constant of cellulase by cellobiose (g/L)
K_{IE}	Inhibition constants of cellulase by ethanol (g/L)
K_{1G}, K_{2G}	Inhibition constants of cellulase and β-glucosidase, respectively, by glucose (g/L)
k_U'	Maximum furfural conversion rate (g/L-h)
$K_{U,U}$	Furfural saturation constant (g/L)
K_Z	Xylose saturation constant for the microorganism (g/L)
K_{ZTA}	Inhibition constants of xylose utilization by acetic and lactic acids (L/g)
L	Concentration of lignin (g/L)
n	Diauxic Phenomenon (Cofermentation) Term (g/L)
R	Concentration of glycerol (g/L)
r_1	Volumetric rate of cellulose hydrolysis to cellobiose (g/L-h)
r_2	Volumetric rate of cellobiose hydrolysis to glucose (g/L-h)
r_3	Volumetric rate of cellulose hydrolysis to glucose (g/L-h)
r_H	Volumetric rate of HMF conversion (g/L-h)

r_U Volumetric rate of furfural conversion (g/L-h)
r_{x1} Volumetric rate of cell mass production from glucose (g/L-h)
r_{x2} Volumetric rate of cell mass production from xylose (g/L-h)
r_{x3} Volumetric cell mass reduction in continuous train's first fermenter (g/L-h)
r_{x3} Volumetric cell reduction term (g/L) (not time dependent)
T Concentration of xylitol (g/L)
t Time (h)
U Concentration of furfural (g/L)
V Fermenter Volume (L)
X Concentration of cell mass (g/L)
Y_{XG} Yield coefficient of cell mass from glucose (g/g)
Y_{XZ} Yield coefficient of cell mass from xylose (g/g)
Y_{RG} Yield coefficient of glycerol from glucose (g/g)
Y_{RZ} Yield coefficient of glycerol from xylose (g/g)
Y_{TZ} Yield coefficient of xylitol from xylose (g/g)
Z Concentration of xylose (g/L)

Greek symbols
λ Rate of decrease in cellulose specific surface area
μ_{m1} Maximum specific growth rate of the yeast, when grown on glucose (h^{-1})
μ_{m2} Maximum specific growth rate of the yeast, when grown on xylose (h^{-1})

Subscripts
T Total value
0 Initial value

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3.0 Process Development Unit

Preparations for corn fiber work began in late 1994 with the first test occurring in February using the PDU's Sunds reactor for pretreatment. Two short runs occurred in March and April, the first using the Sunds reactor and the second run using the Amoco Pretreatment Reactor (APR). All these early experiments used the non-recombinant strain 1400 yeast and the simultaneous saccharifrcation and fermentation (SSF) process.

During redefinition of phase III of the CRADA in the summer of 1995, five tasks were defined for the PDU, as well as a testing program for the APR. The first task was to complete check out activities in the PDU and modify equipment for extended and continuous operation using the APR to pretreat corn fiber. This work was completed in October 1995. The second task was a four-week run of the PDU designed to test the modified equipment and bring the PDU to full operability and began in November 1995. Both the APR and fermentation systems were operated continuously on corn fiber and glucose was fermented to ethanol by 1400. APR testing occurred between the second and third tasks with the goal of identifying satisfactory pretreatment conditions at high enough throughput rates so that reasonable residence times could be used in the 9000-L fermenters. Batch fermentations were conducted in the larger PDU fermenters (1450-L and 9000-L fermenters) to establish the viability of the recombinant organism LNHST2 during the third task beginning in January 1996. This was the first use in the PDU of the recombinant organism designed to co-ferment glucose and xylose using SSCF. The fourth task was a six-week long run beginning mid-March designed to demonstrate continuous operation using LNHST2. Additionally, 6 tons of solid product were collected for animal feed testing. The fifth and final task began in May and generated another 6 tons of solid product and additional process performance information. All PDU runs and APR testing results were documented and these reports are contained in Appendix A-2.

The following sections summarize PDU equipment operation, APR performance, fermentation performance, solid product recovery, predictions of the PDU kinetic model, and an overall performance summary.

3.1 Equipment and Operation Summary

The PDU is an integrated pilot scale system for converting biomass to ethanol with the nominal capacity of one ton of dry feedstock per day. The system is comprised of equipment for feedstock handling, size reduction, prehydrolysis, fermentation, distillation and solid-liquid separation. The PDU is supported by utilities for steam, cooling water, chilled water, hot water, plant and instrument air, nitrogen and deionized water. Size reduction was not necessary for the corn fiber or corn fiber/corn screenings blend; the feedstock was provided pre-milled in plastic or steel drums. Amoco provided their own proprietary reactor process utilizing the Amoco Pretreatment Reactor (APR). The APR was installed in the PDU and outfitted with instrumentation and equipment to integrate it with the existing PDU equipment.

Since the Amoco CRADA work was the first program to be run in the PDU, some of the systems underwent multiple improvements during the seven PDU runs. Those improvements, along with notable operation highlights for each system are outlined below. Experimental results for the various unit operations are provided in Sections 3.2 through 3.4.

3.1.1 Pilot Plant Configuration

This section provides a brief description of the PDU as background on the overall process. A simplified process flow diagram for the PDU with the APR is shown in Figure 3.1.1. The figure shows the overall flow path and equipment in the PDU. Corn fiber is dumped into the main feed hopper (SH120) and conveyed to

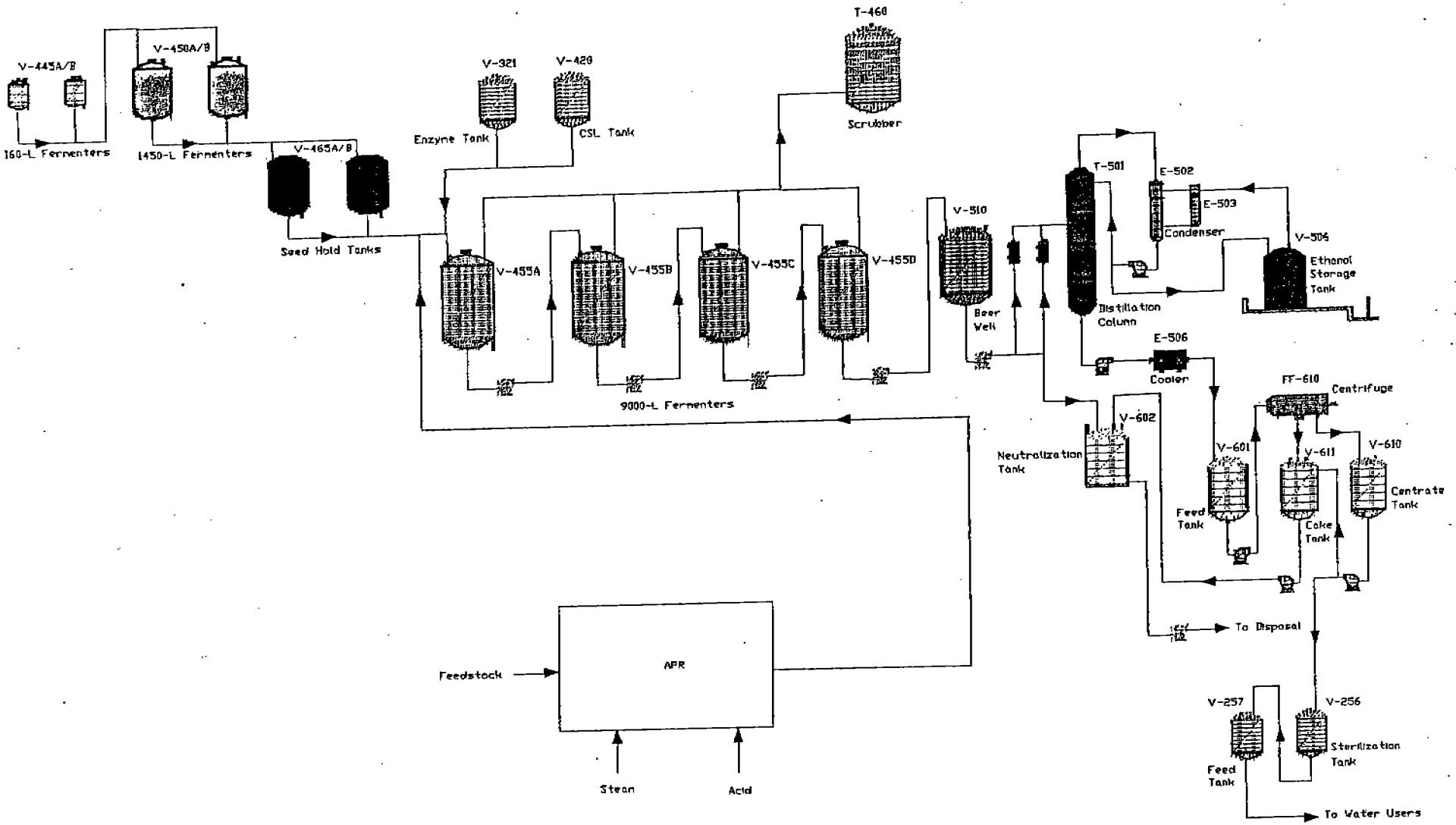

Figure 3.1.1. PDU Process Flow Diagram

the APR through a series of conveyors (weigh belt, cleated, flex screw). It is pretreated by the APR and then pumped to the 9000-L fermenters. In the first 9000-L fermenter, pretreated corn fiber is combined with inoculum, cellulase and glucoamylase (in the case of corn fiber with corn screenings) enzymes and Corn Steep Liquor (CSL) (V420). The fermentation microorganism is started in a small shake flask and successively transferred to a larger shake flask, a 20-L fermenter, 160-L fermenter (V445A/B), 1450-L fermenter (V450A/B) and finally to either a seed hold tank (V465A/B) for continuous inoculation or directly to the first production fermenter (V455A) for initial or intermittent inoculation. Once the first 9000-L fermenter in the tram is filled, fermentation broth is pumped from it to the next 9000-L fermenter (V455B) in the train. Each of the remaining 9000-L fermenters (V455C) receives feed from the preceding fermenter. The level in each fermenter is controlled to maintain a desired residence time. Exhaust gas from the fermenters is sent to a scrubber (T460) to remove volatile organics and odors. The beer well (V510) receives and holds spent fermentation broth from the last fermenter. The fourth fermenter (V455D) was not needed to achieve the desired total fermentation residence time.

Fermentation broth in the beer well is pumped to the distillation column (T501) for removal of the ethanol. Partially purified ethanol from the column is condensed and sent to the ethanol storage tank (V506). The stream from the bottom of the column (containing the solids) is cooled (in E506) and pumped to either the centrifuge feed tank (V601) or the kill tank (V455D) if the fermentation broth contains recombinant organisms. This material is centrifuged to remove the solids, which collect in the cake tank (V611). The liquid fraction from the centrifuge is collected in the centrate tank (V610); this liquid can be sent to the neutralization tank for disposal or returned to the system as recycle water. The recycle portion of the PDU process was not used for the CRADA runs.

3.1.2 Feedstock Handling

Because the APR was not originally in the PDU, an early priority was getting feed to it. Manual drum dumping into the APR main hopper was replaced with automated feeding from the PDU system once a flexible screw conveyor was installed. This allowed the PDU feed system to respond to level sensors in the APR hopper. Manual loading of the PDU feed hopper was still necessary, but was less time consuming because 4 drums could be dumped at one time using the hydraulic dumper. The PDU feed hopper was enclosed more completely and a dust hood added to eliminate any nuisance dust during feedstock charging. The dust hood proved unnecessary for the corn fiber. During Task 4, a vibratory shaker screen was put into the feed conveying system to remove pallet wood and other debris that was found on occasion.

The most labor intensive activities of the Phase 3 runs were receiving and handling the feedstock. Frozen corn fiber in drums was delivered each week in a refrigerated trailer. The drums were off-loaded into covered storage on-site at NREL. At times the feedstock was not fully thawed by the time it was needed, so extra measures were taken to thaw it. During the early runs, nearly 1 full-time person was needed to keep a ready supply of thawed feedstock.

3.1.4 Fermentation

Of all the PDU systems, the fermentation system underwent the most refitting. Many original control or measurement devices either did not work or were incorrectly sized for the actual flow rates. The enzyme, nutrient and inoculum delivery systems were all scaled down in pipe size and flow range for the sensing elements. All of the transfer systems were repiped in an attempt to eliminate dead spots that could harbor contamination.

The production train received a continuous inoculation during Task 2. Bringing the entire seed train up from shake flask to 1450 L proved very manpower intensive, so a method, termed fill and draw by team members, was developed to avoid this. This method consisted of leaving 10% of the inoculum in the 1450 L vessel and adding concentrated, sterile media and sterile water to it. The concentrated media exhibited a tendency to foam during batch sterilization, causing plugging of the vessel exhaust filter. It also plugged the 0.5 inch transfer valve on the 1450 L vessel regularly. The yeast exhibited an increasing lag in growth after 3 successive fill and draws. Inoculum production out paced need due to the minimum working volume of the seed fermenters, resulting in excessive hold times of seed, raising viability questions and dumping of extra seed. The inoculum transfer system, consisting of a Baumann control valve and Micro Motion flow meter, worked well, controlling the inoculum rate within +/-10% of the setpoint. In Task 4, continuous inoculum addition was discontinued because it was determined that the cell population could be maintained in the production train with only an initial inoculation.

Several pH control strategies were tested on the smaller fermenters until a combination of sensing and timed addition/mixing control was chosen. The small size of the seed fermenters required fine control to avoid over addition of caustic.

Corn Steep Liquor (CSL), added to the fermentation as a nutrient, arrived at the PDU in steel drums. CSL typically contains a high microbial load and required sterilization prior to addition to the fermentation. The PDU has a batch sterilization tank (V420) which uses direct steam injection and jacket cooling. Early tests with the CSL, which has 50% solids, showed that dilution was necessary prior to sterilization to avoid burning it. An 18% solution of CSL/water gave passable results, although the CSL still appeared somewhat burned and coagulated. The lowest sterilization time that resulted in sterile CSL was used to avoid overcooking. This may have been in part the cause for contamination found in the CSL tank in later runs. It is possible that a new lot of CSL had either a higher microbial load from the supplier or grew up after being stored at warm temperatures in the PDU.

Another problem encountered during batch sterilization was foaming, at times so great that foam overflowed into the exhaust system. A high temperature, short time (HTST) continuous sterilization system may reduce the coagulation and degradation effects. The CSL batching tanks required a thorough cleaning with both caustic and acid washes between batches. Solids collected on the head plate of the vessel and had to be scrubbed off to avoid contamination of the new batch. The CSL transfer system underwent several changes, finally culminating in a peristaltic pump to meter the addition. A Micro Motion flow meter was used to monitor the CSL flow rate, but not control the pump speed. The peristaltic pump worked well for the CSL.

Cellulase added to the fermentation was originally batched into a support vessel (V321) and transferred to the first fermenter through a variety of methods. A control valve originally installed was too large for the required flow; a small positive displacement pump (MicroPump) provided good flow control when coupled with the Micro Motion meter. Solids in the enzyme occasionally caused erratic meter readings. Due to the low addition rate, a carboy and peristaltic pump were eventually used in Tasks 4 and 5 to meter the cellulase into the fermenter through a septum. Glucoamlyase, also used in these runs, was added once every 24 hours to the first fermenter due to the small amount required. Cellulase sterility was a continuing problem. The supply vessel was sterilized before adding the enzyme, however, due to its heat lability, the enzyme could not be heat sterilized. A 0.22 micron filter, added in-line in Task 2, plugged rapidly. An antibiotic, Nisin was added to the enzymes in Tasks 4 and 5. This appeared to be the best bacterial contamination control strategy.

Transfer lines between the seed fermenters and from the support tanks to the first fermenter appeared to be partly responsible for the contamination problem. Even after piping changes were made to eliminate dead spots, the sterility of the transfer lines was suspect. Clear elbows and transition pieces as well as more break points to aid in cleaning and inspection are recommended. Diligence in cleaning and sterilization of these lines is critical.

Achieving an initial, well-mixed slurry in the first fermenter was difficult due to unhydrolyzed solids in the tank prior to enzyme addition. The vessel was filled with water and sterilized, then the water was drained to the appropriate amount to attain the correct solids concentration at the working volume desired. In Tasks 4 and 5, the pH of the water was adjusted to 11 to help speed the neutralization of the initial solids added to the vessel. Agitation was started when the level in the fermenter reached the agitator blades and neutralization was started. A sterilizable, retractable pH probe installed in this vessel allowed operators to clean the probe of solids that had accumulated during the initial filling. pH was confirmed by off-line samples. In future design, the probes should be located off of the vessel floor and in an area of high turbulence to minimize fouling.

Exhaust gas meters on the main fermenters were originally oversized and incorrectly installed. Back pressure valves on the fermenters were not specified as a tight closing valve; consequently, it was difficult to maintain positive pressure in the vessels without added overlay air. The valves were retrofitted, however, a better-closing class of valve should be used in future installations.

Load cells installed on the main fermenters for level control worked very well once the fermenters and transfer pumps under them were allowed to move by replacing the rigid connections with flexible ones.

Cleaning the fermenters was difficult due to build up of a brown residue on the stainless steel walls. Spray balls and jets helped reduce the manpower required to clean the larger tanks. Even after the residue was removed, the stainless remained discolored. A CIP system was ordered but not available for Phase 3. Two chemical cleaners, one caustic based and one acid based, were used in a cleaning sequence that included a power-sprayer pre-wash, hot caustic cleaning/soaking followed by a rinse and a similar step with acid. Smaller tanks were filled with hot solution and held, usually overnight. Between seed batches in Task 2, the fermenters were usually only rinsed.

Agitation power was an issue in the 9000 L fermenters. The agitators are equipped with 30 hp motors and variable speed drives. The agitator in the first fermenter had to be operated at top speed (125 rpm) in the beginning of a run to keep the solids suspended and the temperature and pH probes uncovered. This top speed does not mean 30 hp use. Power translates to 12 hp/1000 gal. fermenter volume. As the fermentation broth in the first fermenter thinned, the speed was reduced slightly. The agitators in the second and third fermenters ran at 75-100 rpm at all times since the broth was always relatively thin. There is a note on the back of the original--not sure where it goes

A white crystalline solid, identified as sodium carbonate, appeared on the uncovered agitator blades and shaft in 455A during Task 2 and again in Task 4 and 5. This solid formation is most likely the result of the sodium hydroxide reacting with the carbon dioxide in the headspace of the fermenter. The extent of the buildup led to concerns about the load on the motor and shaft seal. The level in the vessel was raised in an attempt to dissolve the carbonate. This worked well enough to continue the run, however chunks of this material caused plugging problems in the fermenter pumps and distillation column piping. Adding the hydroxide below the surface of the broth should eliminate this problem in the future. Another effect of adding the hydroxide through the top of the vessel was slower mixing of the caustic into the broth, causing concerns about the localized effect of high pH on the cells.

To adjust the solids concentration in the fermentation, water was added to the first fermenter through a filtration system. This system was tied into the Sunds pretreatment system and had to be operated manually. A hand diaphragm valve and Micro Motion flow meter allowed operators to regulate flow and monitor flow rate.

A Niro® skid-mounted filtration system was tested during Task 5 on fermentation broth. A summary of the testing can be found in Appendix A-2.7 Task 4 Run Report.

3.1.5 Distillation

The distillation system ran the best of all PDU systems, performing beyond its design capabilities. Only minor fixes were required to bring this system on-line. Plugging of the feed and product piping and the heat exchanger tubes was the most frequent cause of downtime, but was easily cleared with water through flushing ports. There was a direct correlation between the success of the pretreatment in breaking down the solids and the plugging of the column components. Some fouling of the preheaters was seen, but cleaned easily with caustic. Several of the top sieve-type trays plugged once during the runs, requiring hand cleaning through the inspection ports. The bottoms pump seal was replaced twice due to leaking during Tasks 4 and 5, presumably due to the abrasive nature of the bottoms stream, which contacts the seal. In this application, the pump probably requires seal water. Feed rate to the column could be maintained at 6 gpm (design rate was 5 gpm) and sometimes up to 7 gpm. Ethanol concentrations in the overhead varied from the target of 40% up to 73%

throughout Tasks 2-5. Bottoms ethanol concentration was typically less than 0.05%. DACS control of the column was good, requiring little operator monitoring during steady state operation.

During Tasks 3, 4, and 5, where the Recombinant Yeast LNHST2 was used, the distillation column was part of the inactivation (kill) process for the microorganism. In an effort to streamline this process, the bottoms cooler was bypassed so the bottoms was close to 90" C entering V455D.

Ethanol produced in Task 4 and 5 was recycled back to the first fermenter in an effort to control contamination with a higher ethanol level. Due to safety concerns, the 40% ethanol was diluted with water prior to exiting the distillation area into the PDU.

3.1.6 Deactivation of the Recombinant Yeast LNHST2

Since Phase 3 of the CRADA work involved planned use of recombinant organisms in the PDU, part of Task 1 focused on obtaining a Biosafety Level 1 Large Scale (BL1-LS) rating for the PDU operations and facility. The PDU facility was already designed to achieve BL2-LS rating, so work focused on containment methods and the validation of a kill system to deactivate the yeast in the process fluid prior to discharge to the sewer. Since a dedicated kill system was still in the design stage, an interim system using the last 9000L fermenter, was designed and validated during Task 2. The bottoms stream from distillation flowed to this vessel prior to centrifugation so that further handling didn't have to be in a contained manner. The deactivation step originally called for a hold of 60 minutes at >=125° C. A less extreme process involving distillation and a hold of 60 minutes at >=80° C was demonstrated in Task 4 to be adequate to inactivate the yeast. This procedure received temporary approval of the Institutional Biosafety Committee (IBC) and was used for the remainder of Phase 3 (Appendix a-2.14). All possible scenarios for spills or decontamination of process fluid from any part of the fermentation train was taken into consideration and several ports were added to allow transfer of fluid into and out of the last fermenter easily and in a contained manner. BLl-LS approval was obtained in January, 1996.

The most laborious yeast-containing stream to deactivate was the flush water from cleaning the distillation system. Flush water was allowed to accumulate in the distillation sump; bleach was added to kill the cells, then the water was pumped to the neutralization tank. A chlorine monitor is recommended for validating chemical deactivation.

3.1.7 Centrifugation

Testing on the centrifuge began in Task 3 and continued in Task 4. During the initial testing, the centrifuge shut down several times due to high torque between the bowl and the conveyor. Modifications were made between Tasks 4 and 5 to solve this torque limitation. A larger backdrive and motor (7.5 hp compared to 3) and the corresponding larger clutch was installed. The speed of the backdrive was unchanged. This modification allowed the centrifuge to run at a higher torque, but did not affect either the solids recovery or moisture.

A higher backdrive speed gave a low differential speed and thus the driest cake. The feed rate was varied at the start of the run then set at the optimum rate, 2 lpm, which gave good results and did not flood the machine. The deactivated, distilled broth was pumped to the centrifuge with P455D, the positive displacement pump located under fermenter V455D. This pump provided a stable feed rate. A ball valve was originally specified to control the feed rate, but plugged too easily. Feed temperature was tested as a variable, but the 30 to 70°C test range did not result in an appreciable difference in either moisture or recovery.

Solids collection was hampered by plugging in the centrifuge about once per shift. It appeared to be caused by fine solids collecting in the conveyor around the dam, which effectively stopped the centrate flow out of the dam and directed it into the solids chute. Several techniques were used to unplug the centrifuge including stopping feed, adding water, or stopping the centrifuge and hand-cleaning it.

3.1.8 Data Acquisition and Control System (DACS)

Overall, the DACS system supplied user-friendly, automated control of about 80% of the PDU equipment. The system was in a constant state of upgrade throughout Phase 3 in response to operator requests for fixes and improvements. The fermentation control system received much of the focus in an effort to improve the integrated control of temperature, pressure, pH and several other process parameters critical to the success of the fermentation.

The most serious problems in Phase 3 occurred during periods when either the DACS system or programmable logic controllers, for various unknown reasons, reset some part of the PDU equipment including set points in control loops, valve positions and power to motors Faulty wiring in control cabinets, in the I/O cabinets to and from the DACS and software logic glitches are blamed for these resets, which appeared to increase in frequency and severity as Phase 3 progressed. Other problems, such as blown fuses and momentary power outages, were a nuisance but not a long term problem. The operators became adept at identifying and recovering from a control system upset with the help of alarms on the fermenter panels.

3.1.9 Utilities

Extensive work was planned to upgrade the PDU utilities independent of the Amoco CRADA and the timing of several of these projects coincided with Task 1. The most major upgrade was to the Cooling Water (CW) system and necessitated erection of 2 mezzanines to hold the equipment. The new CW system delivers 4 times the capacity and 3 times the heat removal.

A new air compressor was also installed during Task 1 to provide the PDU with its own air supply. A separate Champion compressor provided air to the APR with a backup Champion unit provided temporarily free of charge onsite due to problems experienced with the original unit.

A chemical storage shed was located on the north side of the building, under the CW tower mezzanine. This shed improved efficiency in handling the chemical inventory. The shed has a HVAC unit for climate control and separate containment dikes for acid and base. Early in Task 2, the sodium hydroxide in the PDU froze due to improper handling and storage.

A system was installed to provide hot (150 °F) water to the plant floor for washing, batching, etc. This unit reduced energy usage as it is a more efficient way to heat water than in a jacketed vessel. Another worthy addition to this system would be several hose stations spread throughout the PDLJ to improve washdowns.

Back-up power for the PDU was provided by a generator installed in January, 1996. An Uninterruptable Power Supply (UPS) for the DACS was also installed. Neither of these systems was needed during Phase 3.

The main PDU boiler operated reliably during Phase 3, with minor only leaks and malfunctions. When it was down, the steam generator was a fairly reliable backup.

The deionized water system capacity was doubled to reduce the frequency of recharging the cylinders.

The scrubber proved to be the most time consuming support function throughout Phase 3 runs, in spite of efforts to improve it. DACS monitoring helped reduce trips to the unit, but still required frequent replenishing of bleach and caustic, which had to be diluted to 25% due to the scrubber piping, and the bleach. At the conclusion of each run, the scrubber had to be flushed with phosphoric acid to clean the packing.

During Phase 3 of the Amoco CRADA, the PDU became operational and achieved the goals of the CRADA runs. It has undergone significant improvements in response to the CRADA, becoming a flexible and reliable tool in the biomass to ethanol commercialization effort.

3.2 APR Performance

The development of the APR was a key accomplishment of Phase 3. This section contains a complete account and thorough discussion of the work conducted on the APR thoughtout Phase 3.

3.2.1 Objectives of Pretreatment

The objectives of pretreatment are to:

1. hydrolyze starch and hemicellulose to soluble form,
2. convert soluble glucose and xylose to monomers,
3. make the remaining insoluble cellulose as reactive to enzymatic attack as possible.
4. avoid the formation of compounds that inhibit either enzymes or fermentive organisms,
5. break up the fibrous structure of feeds so that slurries of the substrate have low viscosity

It is desirable to have a continuous (as opposed to a batch) process, to have high throughput, and to minimize (a) dilution of the feed by water or other liquids, (b) the use of chemicals, (c) necessary operator intervention and maintenance, and (d) capital and operating cost.

Pretreatment has typically been the Achilles heel of processes for the conversion of lignocellulosic feedstocks to ethanol. The Amoco Pretreatment Reactor (APR) was developed to meet these objectives with a wide variety of such feedstocks.

3.2.8 Analytical Results

Appendix Q4 (A-2.12) gives the results of the analysis of the product slurry samples. Most values in the table were generated by the NREL PDU support staff, although the analysis of samples (APR-205 through 209) were made by the Amoco Analytical Group in Naperville. Not all samples taken were analyzed, and only those analyzed are included in this table. The following items are included:

- Sample Number, given as APR-xxx.
- % Biomass, the solids in the slurry sample measured by oven drying.
- Sugars Available, the weight percent of each major sugar in the feed on a dry basis.
- Monomeric Sugars in the product slurry on a dry basis, and as a percent of the sugars available in the feed.
- Soluble Sugars (which includes both the monomers and soluble oligomers) in the product slurry on a dry basis, and as a percent of the sugars available in the feed.
- The ratio of Monomeric to Soluble Sugars.
- Concentrations, as weight percent of dry feed, of Hydroxymethylfurfural, Furfural, Acetic Acid, Lactic Acid, Glycerol and Xylitol.

There are several concerns with the values reported in this appendix. First, the concentrations are determined in the laboratory based on the injected samples, which are the liquid portion of the slurry samples. HPLC results are normally given as g/ml. In order to convert these numbers to useful data, it is necessary to know the fraction of the sample that is insoluble solids. Insoluble solids was not always determined, but had to estimated based on the limited number of values available for each block of samples. In some cases, there were no reasonable estimates available; such values are shown in the table in shaded blocks, and represent g/ml rather than weight percent of solids.

A second concern is the accuracy of the HPLC method used to collect most of the data at NREL. The values in the table were typically obtained by use of a Bio-Rad lead column, made to separate weak acids and run at low pH. This technique is called ion-moderated partition chromatography, and is the ethanol industry standard. The column runs hot, and utilizes a refractive index detector. This detector sees everything, but is not very sensitive or specific. This method of operation gives good separation of sugars, but the peaks are broad. Monomers come off last, and the separation of the early peaks is limited. One advantage of the method is that it analyzes for alcohols and acids in the same run it analyzes for sugars.

However, NREL has also used the Dionics method of HPLC analysis, and has adopted it as the standard for mass balance periods where accuracy is most important. This technique is true ion chromatograpy, and runs

at pH = 12, where the sugars become anions. The detector uses pulsed ampherometry and a gold electrode; a voltage pulse oxidizes the anions and the resulting current is measured. Two additional pulses of voltage continuously clean the detector, about once per second. The benefits of this method are narrow peaks and selective detection; this combination insures against interference by non-sugar compounds and increases sensitivity by about a factor of 1000 over the Bio-Rad method. In addition, it allows much more rapid sample injection, typically about 17 minutes (injection to injection) if cellobiose is measured (and cellobiose easily doubles the time needed compared to measurement of only monomers), versus 45 minutes to an hour per sample for the Bio-Rad column. There seems to be very little justification for continuing to use the Bio-Rad technique.

Another concern with the analytical data has been the measurement of the low levels of furfural and HMF in the product. Values of zero have frequently been reported when it was obvious that pretreatment conditions were severe enough to produce at least low levels of these inhibitors. During Task 5, methods were introduced by NREL Analytical that lowered the detection limit and solved this problem, but earlier values of the concentration of these inhibitors are suspect, particularly zero values.

The YSI monomeric glucose values measured by the APR operators have been given in the individual run reports, and will not be repeated here. However, the relationship of the measured YSI values to HPLC analysis is examined in Figure 3.2.8. The relationship between the two methods of analysis is quite strong for each of the three tasks shown (YSI glucose was not commonly taken in earlier runs), but the relationship changes between tasks. The relationships shown are a linear one for the APR Task, but power-law curves for Task 4 and Task 5. The power-law fits are probably not justified over linear relationships, but they do permit the correlations to go through the origin in this case. Whatever type of correlation is assumed for each Task, it is clear that the relationship between YSI and HPLC glucose changed from Task to Task. It is less clear why this is so.

3.2.9 Effectiveness of Pretreatment

The objectives of pretreatment were defined in section 3.2.1. A key concern is how to tell when pretreatment has been effective, that is, what measures define well-pretreated substrate. The fifth objective, breaking up the fibrous structure of the feed, is generally judged by the operators on the basis of appearance, color, feel and smell of the product slurry. This has proven to be quite a reliable indicator that pretreatment has been at least minimally effective. That is, it is a necessary, but not sufficient, indication that pretreatment is effective.

The first, second and fourth objectives are relatively straightforward to measure; the conversion of starch and hemicellulose to soluble form, the conversion of soluble glucose and xylose into monomers, and the possible presence of inhibitory compounds can be monitored by HPLC. Only monomeric glucose is monitored without laboratory analysis via YSI. However, monomeric glucose, like the physical observations of the operators, is only a necessary (and insufficient) measure of pretreatment effectiveness.

The third objective, making the insoluble cellulose reactive to enzymatic attack, can also be determined in the analytical laboratory, but the tests required are more extensive (and therefor expensive) than most analytical evaluations. During the entire Phase 3 program, NREL ran solids reactivity only 5 times. Unfortunately, this measure is the real test of pretreatment effectiveness.

Results reported in the Task 5 Final Report raised doubts about some of these specifications of pretreatment effectiveness. For the first time in Phase 3 work, there was evidence that xylose oligomers were converted to ethanol during SSCF. If Xylose oligomer can be converted to monomer during SSCF, then converting the oligomer to monomer during pretreatment becomes less important.

On the other hand, Phase 3 results suggest the specified level of acetic acid is too high to tolerate. The level was established as tolerable for the conversion of glucose to ethanol in SSF at pH = 4, and for 25% solids. The LNHST2 yeast will indeed ferment glucose under these conditions, but xylose fermentation is more sensitive to acid levels.

Benchtop tests, and the results of Task 5 in the PDU, showed that 25% solids in SSCF leads to very little xylose conversion. Process spreadsheet modeling suggests the cost for ethanol might be $0.75/gallon if high conversions of xylose could be achieved at 25% solids. If the solids level has to lowered to 20% to get high xylose conversion, the cost rises to $0.85/gallon, and if only 15% solids can be tolerated, ethanol would cost $0.97/gallon, all else being equal. So it is critical to determine the relationship between xylose conversion, ethanol and acetic acid levels.

Measures of pretreatment performance are
soluble xylose concentration, monomeric/soluble glucose, monomeric/soluble xylose and acetic acid concentration. A least-squares fit to run time is shown for each data set, and each measure is compared to the temperature of pretreatment measured by thermocouple 12-2 over the course of each experiment. The following observations can be made:

- Temperatures generally rise through each run. as the operators adjusted it to try to maintain pretreatment quality.

- The data are widely scattered, and run time is not a very good predictor of any of the measures, but trends in the direction the measures vary are clear in most cases. The single exception is soluble xylose in Task 2, where a handful of early data points cause the trend line to show a decrease in the measure during the experiment. Ignoring those few points would result in a nearly constant level of soluble xylose through the run, much like the behavior of soluble xylose in Tasks 4 and 5.
- The efforts of the operators to maintain pretreatment quality seemed to work with the measure soluble xylose. In each experiment, the level of soluble xylose remained nearly constant.
- The other three measure, monomeric glucose, monomeric xylose and acetic acid concentration, fell steadily over the course of both Task 4 and Task 5. They did rise slightly for Task 2. The attempt to maintain pretreatment quality didn't work in the most recent two continuous runs, but did in Task 2.
- The measurement of xylose seemed to improve with passing time. As noted in the APR Task report, xylose levels were over 100% of available at one point. By Task 5, such high values no longer occur. As a result, it is impossible to judge the quality of pretreatment based on the amount of soluble xylose, except perhaps in the case of Task 5. In Task 5, it would have to be judged inadequate, since the average values fall around 75% of available xylose, rather than the target 85%.
- Pretreatment was clearly inadequate during Task 2, where each of the measures monomeric glucose, monomeric xylose and acetic acid concentration are significantly lower than in Tasks 4 and 5. This is undoubtedly why it was possible to not only maintain, but to slightly improve, pretreatment as the run progressed - there was plenty of room to work with.
- Pretreatment in Task 5 was more severe than in Task 4; all measures were higher. At the beginning of Task 5, when the measures were highest, pretreatment was probably sufficient according to the guidelines discussed above. However, the concentration of HMF and furfural were also high at this time, probably too high for successful fermentation.

The question remains whether or not any or all of the measures plotted are necessary in defining quality of pretreatment. The relationship between substrate reactivity to cellulase and the more-easily determined properties of the product needs to be confirmed.

3.2.10 Corrosion

3.2.11 Conclusions

1. The APR is an effective means for pretreating corn fiber and mixtures of corn fiber and corn screenings.

2. The important parameters for pretreatment of lignocellulosic materials are temperature, pH and residence time. None of these parameters was directly measured in the APR (until the last few runs), although they were monitored indirectly.

3. Mechanical problems with the APR and its supporting equipment were identified and eliminated over the course of Phase 3.
4. It is important to good APR operation to maintain a constant feed rate of feedstock, free of major chunks of solids.
5. The measurement of xylose by HPLC, of glucose by YSI and of moisture by IR have all showed inconsistencies through Phase 3. The problems with xylose measurement seem to have been resolved, or at least the values of monomeric and soluble xylose no long exceed available xylose.
6. Two runs approaching 1000 hours were completed on the APR with downtime of 100 hours in the first and 50 hours in the second. Two additional continuous runs were also completed, one of 700 hours and the other of 225 hours.
7. Mass balances have not been closed around the APR. It is possible to estimate the flows of unmonitored streams, but the best method of calculation is not clear. The loss of inhibitors with the flash vapor at the end of the APR are important to know for commercial design, and have not been determined.
8. The objectives of pretreatment are clear, but measures thought to be necessary and sufficient to determine good pretreatment have not been confirmed.

10. Pretreatment quality in the continuous runs was insufficient. Quality of the product improved from Task 2 to Task 4 to Task 5.

3.3 Fermentation Performance

Seven fermentation runs were made in the PDU Phase 3. The first three were carried out in early 1995 and the final four were carried out in late 1995 and 1996 after the Phase 3 scope of work was redefined. These runs are referred to as Tasks 2-5.

3.3.1 Early CRADA Runs

Initial runs in the PDU used the glucose-fermenting yeast 1400 and SSF. These runs were designed more to check out equipment operation and procedures than supply information on fermentation performance. When the recombinant co-fermenting organism LNHST2 developed by Nancy Ho at Purdue University was available, operation was switched to this organism.

The first CRADA runs in the PDU with corn fiber occurred in early 1995. The first two runs (run numbers P950206CF and P950310CF) used the Sunds reactor to pretreat corn fiber. The Sunds reactor operates at lower temperatures (170°-200°C) and longer residence times (5-15 min) than the APR.

A 9000-L fermenter was operated in batch mode with 1400 during the first run and achieved an ethanol concentration of 18 g/L in 40 hours at 10% solids. The second run was used to check out continuous feed additions (e.g., enzyme, CSL, and inoculum) and to operate all four 9000-L fermenters with 1400. Because of problems with the feed systems and difficulties with maintaining continuous operation of the Sunds, the fermenters were primarily operated in batch mode. During these periods, glucose additions were required to maintain the fermentation, so no useful information was generated on fermentation performance. This was the first appearance of significant bacterial contamination that would turn out to be problematic throughout CRADA runs.

The third run (P950425CF) began in April 1995 and was the first run that used the APR to pretreat corn fiber. Three 9000-L fermenters were operated in series in continuous mode using SSF. The seed fermentation tram was operated to supply a continuous inoculum of 1400 to the first 9000-L fermenter. Both the seed tram and 9000-L fermenters ran well. Contamination-free inoculum was produced throughout the run. There were still problems with the feed additions systems that were later resolved during Task 1.

A maximum ethanol concentration of 26 g/L was achieved in the third fermenter during this 10-day run. All of the monomeric glucose produced during pretreatment and some glucose from enzymatic cellulose hydrolysis accounted for all ethanol production. A significant drop in ethanol concentration occurred during the run and was attributed to contamination. Both a *lactobacillus* and ***bacillus*** contaminant were identified, and there was evidence of consumption of arabinose that was also attributed to the contaminants. The run was ended after running for the planned 10 days.

3.3.2 Task 2 Run

Following completion of Task 1 activities, Task 2 (P951101CF) began in November 1995 with the goal of checking out the modified PDU equipment and proving continuous operation. This was a month-long run that successfully demonstrated continuous operation of the APR and three 9000-L fermenters using SSF at a 25% solids concentration. The seed train was also operated to provide a continuous inoculum of 1400. Equipment problems with the feed addition systems were solved, although there were some lingering control problems.

The maximum ethanol concentration achieved was 17 g/L. Ethanol process yields (ethanol produced divided by potential ethanol from starch, cellulose, and galactan) were low (10%25%) because of inadequate pretreatment. Monomeric glucose concentrations were low (less than 10 g/L) and because of the ineffective pretreatment, little of the cellulose was converted to glucose. After Task 2, it was learned that one of the APR screws had broken early in the run (noticed but not detected until the APR was disassembled after the run) and was partially responsible for the poor pretreatment.

This run showed that contamination would be a continuing problem and that early detection and elimination of contaminants was necessary to maintain fermentation performance. The contaminants found during this run preferentially consumed arabinose, producing high levels of lactic and acetic acid. The high acid concentrations in this run stopped ethanol production by the yeast. Several ***lactobacillus*** species were identified, but could not be controlled by lowering pH to 4.0. Raising the temperature in the fermenters temporarily reduced the number of organisms, but also killed the yeast. Heat treating was not an effective long-term control measure. Virginiamycin (Lactrol) at low levels (2 ppm) temporarily reduced contaminant

populations. Although both pretreated feed and CSL were suspected as the source of contaminants, no positive identification was made of a source.

3.3.3 Task 3 Run

The first use of the recombinant co-fermenting organism (LNHST2) at large scale occurred in Task 3 during late January and early February 1996. This run was designed to test the organism's batch performance at large scale using the 1450-L and 9000-L fermenters at a 20% solids concentration. This was also the first run to use a blended feedstock of corn fiber and cracked corn in an 8.5 to 1 .0 wet weight ratio. This increased the glucose content of the dry feed from 30%-35% to 40%-45%. A corn fiber blend was used for the rest of the PDU runs. Improvements in APR performance also produced higher sugar levels than had been seen in previous runs.

Three successful batch runs were conducted in the PDU. The first run in a 1450-L fermenter achieved a final ethanol concentration of 28 g/L, but took 60 hours to consume all of the monomeric glucose. This was significantly longer than bench scale fermentations conducted at identical conditions. It was initially thought that the lag may have been caused by inadvertent damage to the yeast inoculum. However, later CRADA work showed that high furfural concentrations can produced a similar lag. After 5 days, only 66% of the monomeric xylose was consumed.

The second run, also in a 1450-L fermenter, achieved a higher ethanol concentration of 40 g/L and consumed all of the monomeric glucose within 24 hours. However, only half of the monomeric xylose was consumed after 7 days. The same performance was obtained with a bench scale SSCF using pretreated material taken directly from the PDU fermenter before it was inoculated. This proved that bench scale results mimic the results of PDU batch runs.

The best results were achieved in the 9000-L fermenter. The ethanol concentration was 47 g/L and all of the monomeric glucose was consumed within 24 h. After 4.5 d, 75% of the monomeric xylose and 60% of the total soluble xylose was also consumed. The ethanol process yield was 57% (definition is modified to ethanol produced divided by potential ethanol from starch, cellulose, galactan, and xylan), significantly greater than yields obtained during Task 2. Better pretreatment released more soluble sugars and made the cellulose more digestible. The yield was low because of a large concentration of soluble sugars (14.3 g/L glucose primarily as oligomers, 16.3 g/L xylose, half as oligomers and the other half as monomers) and unconverted cellulose left at the end of the fermentation. Xylose oligomers were not consumed or converted to monomers in any of the fermentations. *Lactobacillus* contaminants were detected in the fermenters, but at levels too low to affect fermentation performance.

3.3.4 Task 4 Run

Task 4, a six week run, began mid-March 1996 and produced 6 dry tons of solid product for animal feed trials. This task was a continuous SSCF run using LNHST2 and was conducted in three 9000-L fermenters at a 25% solids concentration. This was the first continuous operation of the PDU with the recombinant yeast strain, and proved that the organism's growth rate was sufficient in the first fermenter (with a 36 hour residence time) so that continuous inoculation was not necessary.

Monomeric glucose was completely utilized during the fermentation, however, little or no xylose was converted to ethanol. Ethanol concentrations reached 45 g/L during the early part of the run in the final 9000-L fermenter, but decreased to 35 g/L as sugar concentrations dropped in the pretreated feed. A decrease in

pretreatment severity throughout the run occurred during Tasks 4 and 5. Ethanol process yields at 40% were low because of no appreciable conversion of xylose. Oligomeric glucose and unconverted cellulose were also present, as was seen during tasks 2 and 3. Shake flask testing at Purdue University showed that the combination of ethanol (at 30-80 g/L) and organic acids (combined acetic and lactic acid at 5-10 g/L) can significantly inhibit xylose fermentation. Acetic and lactic acid concentrations during this run were 3-4 g/L and 2-3 g/L, respectively, when significant levels were not being produced by contaminants. Even after 1000 hours of operation, there was no evidence (i.e., higher yields) of adaptation of the yeast to inhibitors.

The *lactobacillus* contaminant was detected throughout the run, but only two major outbreaks occurred that required the use of antibiotics. The contaminant consumed arabinose, producing lactic and acetic acid, which did not appear to have a major impact on glucose fermentation. The high acid levels (each at 10-20 g/L) produced by the contaminant during these outbreaks would have certainly inhibited any xylose conversion, even if no acetic or lactic acids were present in the feed. The source of the contamination was still not identified during this run.

3.3.5 Task 5 Run

Task 5 began in mid-May and also ran for six weeks and produced another 6 dry tons of solid product. SSCF was used with LNHST2 in three 9000-L fermenters. Performance information was obtained at 15% and 25% solids concentration. The lower solids concentration was done to improve xylose conversion. Again, continuous inoculation was not required.

Concentration profiles of major components during this run (typical of many of the runs) are shown in Figure 3.3.5.1. The figure shows the rise in acetic and lactic acid concentration and corresponding drop in arabinose concentration during periods of heavy contamination. Ethanol concentrations increased during the middle part of the run while xylose concentration. The increased conversion of xylose to ethanol was probably due to the declining pretreatment severity that reduced acetic acid and other inhibitor concentrations. While at the same time, high acetic and lactic acid concentrations produced by the contaminants were dropping because of dilution in the fermenters. The drop in ethanol concentration at 650 hours corresponds to a change from 25% to 15% solids concentration in the fermenters during Task 5.

Two complete mass balances were performed during this run. The first was done at a 25% solids concentration, where 73% of the C6 sugars (i.e., monomeric and oligomeric glucose and-galactose) and 26% of monomeric xylose were converted to ethanol (34% overall conversion of monomeric xylose to ethanol and by-products). The ethanol concentration reached 35 g/L. Later in the run when acid levels were lower (due to wash out of the acids earlier produced by the contaminant), monomeric xylose conversion increased to 50% and ethanol concentration increased to 42 g/L. At the second mass balance point (15% solids concentration), 77% of the C6 sugars and 56% of the xylose were converted to ethanol and total xylose conversion increased to 70%. Process yields for the first and second points were low at 47% and 55%, respectively, because unconverted sugars were leaving the process in the form of cellulose, oligomeric glucose, and monomeric and oligomeric xylose. Further HPLC analysis of the oligomeric glucose showed that about 15%-20% was disaccharides, probably produced by reversion reactions. The rest was identified as an octamer, but the origin (cellulose or starch) is still unknown. Further work would be necessary to determine if these oligosaccharides could be converted to monomeric form.

Table 3.3.5.1 compares xylose conversion from four chemostat runs to PDU data generated during Task 5. The best match is Run 3 data to PDU data at 25% solids and a 72 hour residence time. The xylose conversions are somewhat similar at 31% and 37%, respectively. The problem with this comparison is that differences in pretreatment severity will affect xylose conversion. Pretreatment severity was greater for the pretreated

material used in runs 1-3, which may explain the poorer xylose conversion during chemostat runs. However, in spite of this fact, the chemostat data does provide a good indication of large scale performance at 25% solids.

Table 3.3.5.1. Comparison of Xylose Conversion During PDU Task 5 With Chemostat Data

	Solids Concentration (%)	Xylose Converted (%)	Residence Time (h)
Run 1	35	15	72
Run 2	35	29	72
Run 3	24	31	72
Run 4	21	36	48
Task 5, PDU	25	50	108
Task 5, PDU	25	37	72
Task 5, PDU	15	80	108
Task 5, PDU	15	70	72

A *lactobacillus* contaminant was detected in all fermenters throughout the run. Major outbreaks, characterized by rapidly rising acetic and lactic acid concentrations, were successfully controlled by addition of Lactrol. The high acid levels did not significantly affect glucose fermentation, but did inhibit xylose fermentation. Contamination checks during this run identified two probable sources: CSL and pretreated feed. Both sources were characterized by different *lactobacillus* species that were detected in the fermenters at different times. CSL contamination may be due to inadequate sterilization and cleaning of the tank and transfer lines. The contaminants in the pretreated feed may have been picked up in the flash system and transfer line, since contaminants probably cannot survive pretreatment.

3.4 Solid Residue Recovery

Corn fiber is one of the four products/co-products (the others are starch, gluten and germ) produced in a typical corn wet mill. Mixed with concentrated steepwater (sometimes called condensed corn distillers solubles), corn fiber is sold as corn gluten feed and used as an animal feed for beef and dairy cattle, swine and poultry. Corn gluten feed along with distillers dry grains with solubles (DDGS, the co-product from a typical corn to ethanol dry mill) are sold on the basis of their protein content. Corn gluten feed has a typical protein content of 21%, while DDGS has a typical protein content of 27 % (see Table 3.4.1 for typical analysis of both co-products). The protein that enters the SWAN Biomass process from the corn fiber (and the screenings) will pass through the process unchanged (there will be some losses of soluble proteins) and will exit with the unfermentables as the co-product. Because a considerable amount of the fiber will be fermented, the protein content of the co-product will be higher than corn gluten feed and approximately equal to DDGS and thus should bring a higher price per ton than corn gluten feed as an animal feed.

In March of 1996, a research program was set up to study the processing methods of separation and drying and the animal feed quality characteristics of the SWAN co-product. This plan included the following:

Mass Balance
Whole Stillage Separation
Recycle Studies
Evaporation Studies
Drying Tests
Pelletizing Tests
Animal Feed Tests

The results of these tests and studies carried out to date are included in the following report.

3.4.1 Solids (Co-product) Handling Process

3.4.1.1 Co-Product Handling Process

In the co-product handling process, whole stillage (beer column bottoms) from the beer column contains considerable amounts of valuable protein containing solids that are recovered in the co-product handling area for use as animal feed. These suspended solids are typically separated from the liquid portion (which contains the soluble solids and is called thin stillage) by decanting centrifuges and dried using rotary steam tube dryers. The dry co-product is stored and shipped as a high protein animal feed for poultry, swine and cattle. For a more detailed description of the process see section 4.2 of this report.

3.4.1.2 Co-Product Handling Material Balance

The material balance for the co-product handling process in pounds per hour is given in Table 3.4.2. It is based on the spreadsheet model base case run but with some significant differences. This balance is based on our PDU experience, centrifuge tests by Alfa Laval and Bird Machine and drying tests by Davenport Machine. Much of the experience from these tests have not been included in the spreadsheet model. The centrifuge tests showed an expected maximum decanter output of only 30 % solids. The major effect of this lower than expected separation is a high dry recycle to wet cake ratio (almost 4 to 1) which increases the size and cost of the drying process equipment.

The balance shows that for a feed rate of 750 dry tons per day, the SWAN Biomass process produces approximately 449 dry tons per day of SWAN co-product or a reduction of corn gluten feed production of approximately 40 % from a typical wet mill. Add to this the addition of 39,166 lbs/hr of concentrated steepwater (figures from Pekin Energy and these figures are not included in the spreadsheet model) which adds another 258 dry tons per day, making the total output of the SWAN Biomass process with steepwater at 707 dry tons per day of total co-product. The balance also shows that the protein level of the co-product to be only 18.0%. This is considerably below the 25.5% shown in Table 9 for the co-product taken from the Davenport Machine tests using steepwater. This substantiates the suspicion that the protein level used in the spreadsheet model (7.5%) was too low and should have been closer to 10 or 11% which produces a co-product protein level of about 25 %. This is more logical as normal corn gluten fiber has a protein content of 21% (which includes the addition of steepwater), see Table 3.4.1. Without steepwater the protein level in the co-product would be between 18 and 19%, using the higher starting protein level.

Table 3.4.1

Table 3.4.1

TYPICAL ANALYSIS CORN GLUTEN FEED AND DPGS			
ANALYSIS	CORN GLUTEN FEED		DDGS
DRY MATTER, %	88		92.5
PROTEIN, %	21		27
CRUDE FIBER, %	10		8.5
ASH, %	7.8		4.5
CALORIES, KCAULB	795		1175
THREONINE, %	0.9		0.95
CYSTINE, %	0.5		0.4
VALINE, %	1.04		1.33
METHIONINE, %	0.5		0.6
ISOLEUCINE, %	0.6		1
LEUCINE, %	1.9		2.7
PHENYLALANINE, %	0.8		1.2
HISTIDINE, %	0.7		0.6
LYSINE, %	0.6		0.6
ARGININE, %	1		1
TRYPTOPHAN, %	0.1		0.2
CALCIUM, %	0.2		0.35
COPPER, PPM	10		44.7
IRON, PPM	304		200
MAGNESIUM, %	0.42		0.35
MANGANESE, PPM	23.8		30
PHOSPHORUS, %	0.9		0.95
POTASSIUM, %	1.3		1
SODIUM, %	0.12		0.9
ZINC, PPM	88		85
SULFUR, %	0.16		0.3
SOURCE: REFERENCE #2			

3.4.2 Solid/Liquid Separation

3.4.2.1 Whole Stillage, Thin Stillage and Steepwater Analysis

Table 3.4.3 shows the actual analysis of the whole and thin stillages from the PDU runs. The steepwater analysis was obtained from Pekin Energy and is a typical analysis of their concentrated steepwater.

3.4.2.2 Solid/Liquid Separation Studies

Three vendor studies were carried out to determine the best method (driest cake and highest solids recovery) to separate the solids from the liquid in the whole stillage. These tests are discussed in sections 3.4.3.2.2, 3.4.3.2.3 and 3.4.3.2.4. In a typical dry mill this separation is done by using decanting centrifuges. The PDU used a Sharples P-3000 decanter and its actual performance is discussed first.

3.4.2.2.1 PDU Centrifuge Operations, Results and Improvements

A Sharples P-3000 decanting centrifuge was used in the PDU to make our separations and is represented by the material balance shown in Table 3.4.2. Table 3.4.4 shows the Solids Drumming Log Sheet which summarizes the actual performance of the PDU centrifuge. The main problem with the PDU centrifuge was the low solids content of the wet discharge cake. This averaged 22.67 % for the entire run. Expected values were in the 30 to 35% range. When attempts were made to increase the solids level the centrifuge would shut down on high torque between the bowl and conveyor drives. Alfa Laval and Bird Machine were contacted to develop a plan to improve the wet cake solids content. Between Tasks 4 and 5 (May 24, 1996) modifications were made to the PDU centrifuge. A larger backdrive and motor (7.5 HP compared to the existing 3 HP) and a corresponding larger clutch were installed. These modifications allowed the centrifuge to run at a higher torque, but unfortunately did not affect the moisture content of the cake. The percent solids in the wet cake averaged 22.89% before the modifications and 22.49% after. It should also be noted that the solids in the feed after the modifications did drop to 12.80% from 15.26% and the solids in the centrate dropped to 9.36% from 12.30%. This data would indicate that we did see a slight improvement in the recovery of the total solids (from about 57% recovery to about 66% recovery).

Other variables were evaluated to see if they improved the solids content of the cake, they included 1) feed rate, 2) back drive speed, 3) feed temperature and 4) pond depth. The optimum feed rate was 2 gpm which gave good separations and did not flood the machine. Before the modifications, a back drive speed of 4 was the maximum possible without the machine kicking out on high torque. After the modifications, back drive speeds of between 6 and 7 were tried with the speed giving the best results being 6.5. Shut downs due to high torque were not a problem after the modifications. Feed temperatures were varied from 30 to 70°C with no appreciable differences in moisture levels or solid recoveries. Pond depth was adjusted based on recommendations from Alfa Lava1 and after some experimentation was held constant at a setting of 4.5.

Another problem that was experienced in both Tasks 4 and 5, was solids plugging the centrifuge. This appeared to be cause by fine solids collecting in the conveyor around the dam, which effectively stopped the centrate flow out of the dam and directed it into the solids chute. This problem was never really resolved and had to addressed on a once per shift basis by hand cleaning the chute.

Overall with the modifications, the Sharples P-3000 functioned acceptably as a solids separator.

Table 3.4.2

MATERIAL BALANCE

CO-PRODUCT HANDLING SYSTEM

STREAM COMPONENTS, LBS/HR	1	2	3	4	5	6	7	8	9
	WHOLE STILLAGE	W E T CAKE	THIN STILLAGE	DRY RECYCLE	STEEP WATER	FEED TO DRYERS	DRYER VAPOR	DRYER DISCHARG	CO-PRODUCT
DISSOLVED SOLIDS	25,405	12,409	12,996	191,761	9,792	213,962	0	213,962	22,201
SUSPENDED SOLIDS	21,050	21,039	11	168,016	9,792	198,846	0	198,846	30,830
PROTEIN	4,725	4,725	0	57,798	5,875	68,397	0	68,397	10,600
TOTAL SOLIDS	46,455	33,448	13,007	359,777	19,583	412,808	0	412,808	53,031
WATER	159,775	78,044	81,731	39,975	19,583	137,603	91,735	45,868	5,892
TOTAL	206,230	111,492	94,738	399,753	39,166	550,411	91,735	458,676	58,923
PERCENT DISSOLVED SOLIDS	12.3	11.1	13.7	48.0	25.0	38.9		46.6	37.7
PERCENT SUSPENDED SOLIDS	10.2	18.9	0.0	42.0	25.0	36.1		43.4	52.3
PERCENT PROTEIN	2.3	4.2	0.0	14.5	15.0	12.4		14.9	18.0
PERCENT TOTAL SOLIDS	22.5	30.0	13.7	90.0	50.0	75.0		90.0	90.0
SPECIFIC GRAVITY	1.075		1.038		1.15				
GPM	384		183		68				

ASSUMPTIONS:

1. BASED ON THE ANALYSIS SHOWN IN TABLE 3 SOLIDS RECOVERY IS 72 %
2. WET CAKE MOISTURE CONTENT TAKEN AT 30 %
3. STEEP WATER IS 50% MOSITURE AND 25% DISSOLVED/SUSPENDED SOLIDS
4. FEED TO THE DRYER IS 75% SOLIDS
5. CO-PRODUCT IS 10% MOISTURE
6. WHOLE STILLAGE FIGURES FROM THE 12/2/96 BASE CASE SPREADSHEET RUN
7. STEEP WATER FIGURES FROM PEKIN ENERGY, ANAYLSIS FROM TABLE 3

3.4.2.2.2 Komline-Sanderson Rotary Vacuum Filter Study

The solid/liquid separation plan outlined several other methods of separating the solids from the liquid , one of which was the possible use of a rotary vacuum filter. Komline-Sanderson (K-S)agreed to take a preliminary look at our whole stillage to see if a rotary vacuum filter could be used as a separating device. Using a (A-2.15 is a copy of their report) 0.1 sq. ft. test leaf filter, K-S found that the solids loading for our whole stillage ranged between 1.46 and 3.88 pounds of dry solids per square foot of filtration area per hour. These rates were for drum speeds of 2 to 12 minutes per revolution and for two different filter media's (K-S201 and K-S232). Wet cake solids concentration ranged between 27.39 to 28.57 % with good retention of the suspended solids (filtrate suspended solids were 111 to 305 ppm). These solids loadings were determined to be too low for practical application of a rotary drum vacuum filter (corn gluten for example has a solids loading of between 5 to 6 and a wet cake solids concentration of 35 to 40%).

3.4.2.2.3 Alfa Lava1 Centrifuge Study

Based on the results we were getting in the PDU with the Sharples P-3000 Super-D-canter centrifuge, we asked Alfa Laval to conduct a study for us to determine what solids level we could expect under ideal conditions in the PDU and in a commercial size centrifuge. Initially they centered their study on centrate clarity (miscommunication on the part of the author and not a controlling variable) controlling on a centrate concentration of 12 % total solids but then switched to wet cake solids after further conversations. They were shipped one 55 gallon drum of still bottoms on May 14, 1996. The overall composition of the drum varied somewhat but ranged between 13.0 to 14.0 % total solids which is in the mid range of the first 30 solids drum lots whose feed averaged 13.95 % total solids. Alfa Laval tested a Sharples P-660 Super-D-Canter centrifuge with three different conveyors, (1) plough, (2) BD disc and 3) Kiwi (see A-2.16 for the full report). Feed rates varied from 0.22 to 1.43 gpm with differential speeds (between the bowl and conveyor) of 5.0 to 21 .O rpms. All tests were conducted at a feed temperature of 38°C.

They demonstrated a maximum wet cake % solids of 33.8 % using the Kiwi conveyor and a maximum solids recovery of 94.9 % using the BD disc conveyor. Analyzing the data would lead to the following additional conclusions (see Table 3.4.5):

1. Solids recoveries increase with increased pond depths (centrate % total solids decrease).
2. Increased flow rates decrease recoveries.
3. For a given pond depth recoveries decrease with decreasing speed differential.
4. There is a correlation that would indicate that increase % solids in the wet cake will result in decreased recoveries (see Table 3.4.6).

From this data, it would appear that reaching 35 % solids in the wet cake in a commercial or pilot plant scale decanter with our feed is not realistic. Nor does it appear to be desired as reaching this level would decrease recoveries and send more solids forward in the centrate. Since we will be discarding the centrate (thin stillage) this would be a direct loss of co-product. From the stand point of total solids recovery, the BD disc conveyor, not the Kiwi conveyor as recommended by Alfa Laval, appears to be the best configuration for our decanter. Centrifuging our feed at a higher temperatures than 38°C might help recoveries, since our stillage will leave the beer still at about 115°C, this needs some consideration.

Table 3.4.3

Table 3.43

TYPICAL ANALYSIS		
WHOLE AND THIN STILLAGES AND STEEP WATER		
ANALYSIS	WHOLE STILLAGE (1)	THIN STILLAGE (1)	STEEP WATER (2)
DRY MATTER, %	19.7	7.6	45
PROTEIN, %	5.9	0.8	15.3
CRUDE FIBER, %	2.2	0.1	0.7
ASH, %	1.9	2.1	4.8
CALORIES, KCAL/LB	350	57	373
THREONINE, %	0.23	0.03	0.51
CYSTINE, %	0.12	0.01	0.24
VALINE, %	0.3	0.03	0.54
METHIONINE, %	0.08	0.01	0.15
ISOLEUCINE, %	0.21	0.02	0.44
LEUCINE, %	0.7	0.05	0.97
PHENYLALANINE, %	0.29	0.03	0.44
HISTIDINE, %	0.14	0.02	0.31
LYSINE, %	0.06	0.02	0.62
ARGININE, %	0.07	0.01	0.44
TRYPTOPHAN, %	0.03	0.01	0.08
CALCIUM, %	0.0084	0.0042	0.037
COPPER, PPM	1.56	0.41	1.82
IRON, PPM	42.8	3.67	43.2
MAGNESIUM, %	0.0175	0.0131	0.311
MANGANESE. PPM	1.37	0.52	15.2
PHOSPHORUS, %	0.0785	0.0842	0.821
POTASSIUM, %	0.0637	0.06	1.11
SODIUM, %	0.69	0.7	0.537
ZINC, PPM	14.9	2.41	0.0427
SULFUR. %	0.19	DNR	DNR
SOURCE: (1) CORNING HAZLETON ANAYLSIS DATED 02/21/96			
(2) CORNING HAZLETON ANAYLSIS DATED 01/19/94			

Table 3.4.4

						SOLIDS DRUMMING LOG SHEET						
		DRUMMING INFORMATION						CENTRIFUGE INFO				
DATE	LOT	NO.	PERCENT SOLIDS			DRUM	SULF	FEED	BK. DR.	POND	TOTAL	COMMENTS
	NO.	DRUMS	FEED	SOLIDS	LIQUID	WEIGHT	ATES	RATE (gpm)	SPEED	DEPTH	SOLIDS	
4-2-96	1	6	14.72	22.53	11.32	470	0.63	2	4	4.5	635	DARK LOT
4-3-96	2	8	15.03	21.7	11.06	470	0.73	2	4	4.5	816	DARK LOT
4-5-96	3	2	14.5	28	12	470	0.8	2	4	3.2	263	DARK LOT
4-6-96	4	6	14.66	21.13	11.63	470	0.7	2	4	4.5	596	DARK LOT
4-9-96	5	9	17.17	21.73	14.61	470	0.87	2	4	4.5	919	Reduced kill - product lighter
4-10-96	6	14	15.57	24.12	14.45	470	1	2	4	4.5	1587	
4-13-96	7	6	12.68	20.92	10.02	470	0.7	2	4	4.5	590	
4-17-96	8	11	16.58	24.25	15.46	470	0.73	2	4	4.5	1254	Slow going to drums
4-18-96	9	0	14.33	21.7	9.68	0	0.5	2	4	4.5	0	Initial separation
4-18-96	9	4	10.04	17.15	6.14	470	0.25	2	4	4.5	322	After one wash (1:1 ratio)
4-19-96	10	12	17.85	24.46	14.51	470	0.65	2	4	4.5	1380	Started with temp. @ 48 C
4-22-96	11	9	14.75	21.46	10.97	470	0.65	2	4	4.5	908	Started with temp. @ 48 C
4-26-96	12	8	17.39	24.27	15.49	470	0.88	2	4	4.5	913	Started with temp. @ 48 C
4-27-96	13	4	18.4	27	14.8	470	0.73	1		10	508	Used Bird Centrifuge
5-24-96	14	4	13.72	23.86	14	470	0.6	2	7	4.5	449	
5-24-96	15	8	10.98	21.42	5.99	470	0.5	2	6	4.5	805	
5-26-96	16	7	10.88	19.79	8.51	470	0.6	2	6.5	4.5	651	
5-28-96	17	6	10.93	20.37	7.97	470	0.5	2	6.5	4.5	574	
5-31-96	18	8	12.2	22.02	9.93	470	0.6	2	6	4.5	828	
6-1-96	19	10	14.02	23.55	8.1	470	0.6	2	6.5	4.5	1107	
6-4-96	20	11	14.67	25.1	10.7	470	0.6	2	7	4.5	1298	
6-7-96	21	13	17.56	26.2	13.66	470	0.72	2	6.5	4.5	1601	
6-9-96	22	9	15.16	24.4	11.59	470	0.65	2	6.5	4.5	1032	
6/13/96	23	10	15.7	23.09	13.17	470	0.75	2	6	4.5	1085	
6/17/96	24	8	13.33	22.15		470	0.65				833	
6/18/96	25	9	11.42	21.31	8.27	470	0.55	2	6.5	4.5	901	
6/20/96	26	8	10.14	21.97	7.94	470	0.6	2	6.5	4.5	826	
6/21/96	27	8	10.72	20.45	6.37	470	0.45	2	6.5	4.5	769	
6/23/96	28	8	9.21	22.1	7.93	470	0.4	2	6.5	4.5	831	
6/25/96	29	6	8.6	22	6.2	470	0.5	2	6.5	4.5	620	
6/27/96	30	1	5.5								0	
		99	15.26	22.89	12.30		0.70					BEFORE CENTRIFUGE UPGR
		133	12.80	22.49	9.36		0.58					AFTER CENTRIFUGE UPGRADE
TOTAL/AVG		233	13.95	22.67	10.77	470	0.636				24901	
											22591	EXCLUDING LOTS 1 TO 4

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Table 3.4.5

Table 3.4.5

SUMMARY OF ALFA LAVAL CENTRIFUGE STUDY					
CONVEYOR TYPE	MAX RECOVERY	% SOLIDS AT MAX REC		MAX % SOLIDS	RECOVERY AT MAX SOLIDS
PLOUGH CONVEYOR	85.3	25.9		32.5	17
BD DISC CONVEYOR	94.9	22.3		22.3	94.9
KIWI CONVEYOR	88.6	22.9		33.8	32.3

Table 3.4.6

ALFA LAVAL SHARPLES - LABORATORY CENTRIFUGE DATA							
CONVEYOR TYPE	SOLIDS IN FEED %	POND DEPTH	SPEED DELTA RPM	FEED RATE GPM	WET CAKE SOLIDS %	SOLIDS RECOVERY %	CENTRATE TOTAL SOL %
PLOUGH	14.0	2.00	5.0	0.28	29.5	41.2	12.4
				0.51	32.5	17.0	13.3
				0.89	31.7	9.5	13.7
				1.43	33.0	0.0	14.0
	13.7	2.00	9.8	0.28	29.9	58.5	11.5
			20.8	0.29	28.4	50.8	11.9
				0.58	29.0	33.6	12.5
				0.83	29.3	19.3	13.0
	13.7	3.00	10.0	0.36	28.1	53.9	11.7
			21.0	0.33	28.1	58.8	11.5
				0.56	28.5	28.0	12.9
				0.84	26.0	28.1	12.8
	13.7	3.75	10.0	0.27	26.2	81.5	10.5
			21.0	0.26	25.9	85.3	10.4
				0.58	26.3	43.8	12.2
				0.83	28.0	31.6	12.6
BD DISC	13.0	4.06	10.1	0.43	22.3	94.9	9.8
			20.8	0.33	21.8	94.3	9.9
				0.91	21.5	83.3	10.4
KIWI	13.0	2.00	5.1	0.27	32.4	42.9	11.4
				0.53	32.7	25.8	12.0
				0.75	33.8	32.3	12.2
				1.11	33.7	21.2	12.2
			9.9	0.26	30.3	49.2	11.2
			20.6	0.22	26.6	41.8	11.6
		3.75	5.3	0.25	27.0	80.6	10.0
				0.58	28.3	37.3	11.7
				0.96	29.5	30.5	11.9
			10.1	0.25	25.4	83.6	9.9
			20.8	0.34	22.9	88.6	10.0

3.4.2.2.4 Bird Machine Company Centrifuge Study

Decanter centrifuge studies were also conducted by Bird Machine Company. These tests were conducted in July, 1996 and utilized Bird's model 0100 solid bowl decanter. Unfortunately the initial sample and the drums sent Bird were not representative of the still bottoms produced in the PDU. The sample contained a suspended solids content of only 3.79 % and the drums contained a total solids content of only 5.66 %. These are well below the normal levels of 7 to 10% and 13 to 16%. There are no explanations as to what happen but it would appear that Bird was sent PDU centrate and not feed. However Bird's conclusions, while subject to some question, substantiated the conclusions made by Alfa Laval. These conclusions can be summarized as follows (see Table 3.4.7 for a summary of the Bird data and A-2.17 for the full report):

1. The SWAN still bottoms require long settling times (low flow rates) and a high gravitational force to separate and dewater in a solid bowl centrifuge.
2. Based on Bird's experience the SWAN solids are "much more difficult to handle than the spent grains from a dry corn mill."
3. The dewatered solids using Bird's Model 5100 would be in the 20 to 25% solids range. Solids levels of 30 to 35% would not be obtained on a production size unit.
4. The production centrifuge would have to operate at a 3000 x G level and at a much reduced rate than normal for the Model 5 100.
5. They estimate that solids recovery will range between 25 to 30% (these low figures are most likely effected by the low solids content of the feed they tested).
6. Increased flow rates reduces recoveries.
7. There appears to be a correlation between increased % solids in the cake and decreased solids recoveries.

Bird did generate some useful design information in their study and this information is included in Table 3.4.8. This data includes thin stillage (centrate) pH, particle size analysis of the wet cake solids and the wet cake bulk density.

3.4.2.2.5 Evaporation Studies

In a typical whole grain dry mill, evaporation is used to concentrate the centrate (thin stillage) from the decanter centrifuges to recover the soluble protein and any suspended protein that might pass through the centrifuge. This concentrate is then added back to the wet cake along with any dry solids recycle just before the total mass is fed to the dryers. The resulting dry product is called distillers dried grains with solubles (DDGS). The condensate from the evaporator is either sent directly to wastewater treatment or used in the CIP system. This method of recovering soluble proteins has proven very economical particularly when using either thermal or mechanical recompression for heat recovery in the evaporator. In the case of corn fiber and corn screenings, it was unclear if an evaporator would be necessary. Corn fiber in a typical wet mill goes through several water washing stages (7 to 9 in a typical mill). These washing steps more than likely wash out any soluble proteins (the primary purpose of these washes is to remove combined starch) contained within or on the fiber. Therefore most if not all the soluble proteins have been recovered in the mill water from the washing steps or the filtrate water from the fiber presses.

Table 3.4.3 gives a typical analysis of the thin stillage from the PDU run. It shows a protein level of 0.8% which converts to about 1200 lbs/hr of protein using the spreadsheet model mass balance. This raised the question, at what level of protein loss in the thin stillage would justify installation of an evaporator. To help

answer this question we obtained a quote from Dedert Corporation for a mixed flow quadruple effect thermal recompression evaporator. Dedert quoted an equipment cost of $1.5 million which we estimated to be $3,465,498 installed ($2,887,498 installed cost plus $578,000 for the evaporator building). A discounted cash flow analysis was done to determine the return on investment (ROI) if the above evaporator was installed to recover the 1200 lbs/hr, which is 25.4% of the total protein. Figure 3.4.1 ▪ Evaporator Economics, gives the estimated ROI for this case as 11% which is a marginal return. A protein lost of between 28 and 33% would be needed to justify (ROI of between 15 and 20%) investment in an evaporation system. Given the results of the PDU runs and the data reported in this report, this level of protein loss is highly unlikely (the spreadsheet model predicts a loss of 3.5%) and it was decided to exclude the evaporator in our design.

Figure 3.4.1

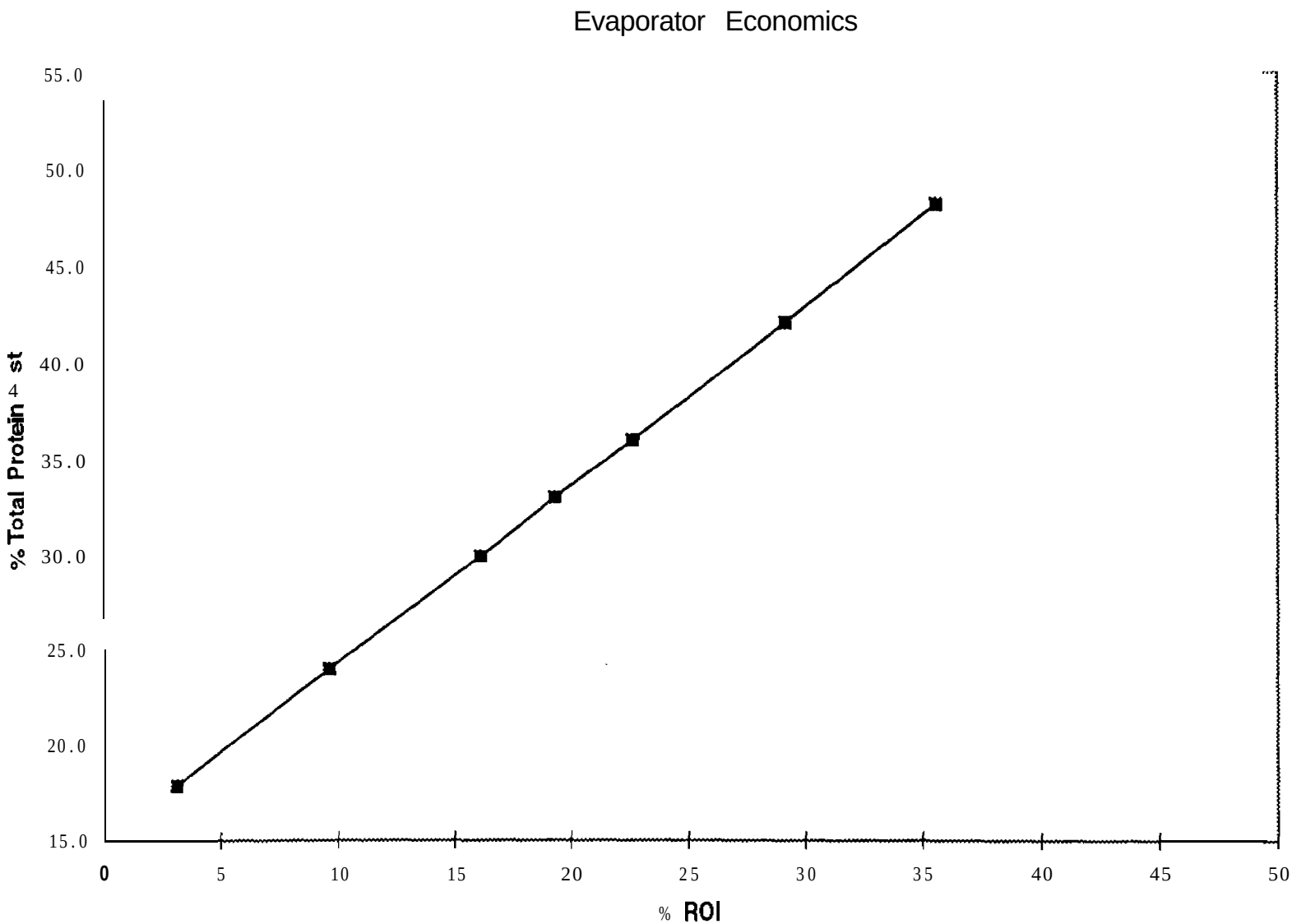

Evaporator Economics
55.0
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
% Total Protein st
4
0
5
10
15
20
25
30
35
40
45
50
% ROI

Table 3.4.7

BIRD MACHINE - LABORATORY CENTRIFUGE DATA							
CONVEYOR TYPE	SOLIDS IN FEED %	POND DEPTH	SPEED DELTA RPM	FEED RATE GPM	WET CAKE SOLIDS %	SOLIDS RECOVERY %	CENTRATE TOTAL SOL %
	5.7	21/32	11.3	0.8	17.7	18.7	5.5
			19.4	2.0	23.5	22.9	5.5
				3.0	21.1	33.9	5.3
				4.5	19.7	24.0	5.4
			26.0	0.2	17.5	42.3	5.2
				2.0	19.5	27.6	5.5
			36.6	2.0	20.8	27.0	5.4

Table 3.4.8

BIRD MACHINE DATA

Thin Stillage (Centrate) pH 5.8

PARTICLE SIZE ANALYSIS OF THE SOLIDS

% Volume	Particle Diameterlum <)
25	11.33
50	28.28
75	69.18
90	140.8
99.9	876.4

WET CAKE BULK DENSITY

% TS in Cake	lbs/ft2
20.8	65.6
16.4	63.1
15.5	62.5

3.4.2.2.6 Recycle Studies

The base case spreadsheet model (see section 4.3) assumes a thin stillage recycle (called back set) of 26.6% of the total thin stillage produced. Thin stillage is normally recycled in most ethanol plants to reduce water usage and waste treatment costs. The base case spreadsheet model shows a total water usage of approximately 13.6 gallons of water per every gallon of ethanol produced. While this adds only about $0.010 per gallon of cost for water purchase the real cost addition is from wastewater treatment costs which can run to many times this cost. Thus it is important that as much of the thin stillage be recycled as possible keeping in balance the build up of unacceptable contaminates that could inhibit fermentation. These contaminates include acetic acid and various salts. The corn to ethanol industry has experienced inhibitions from salts build up from the backset and have normally limited this recycle to 25% in most cases with a maximum of 50% being recycled. No recycle studies have been conducted to-date in the PDU or lab and these should be planned for the next PDU runs regardless of the feedstock being studied.

3.4.3 Co-product Drying

In order for the coproduct to be acceptable to the animal feed market it had to be dry and in a form that would be easy to blend with other feed components. Vendor tests were conducted to determine the proper equipment and costs for making an acceptable product.

3.4.3.1 Davenport Machine Work

One of the basic assumptions of this study was that the existing dryers at the Pekin Energy Plant in Pekin, Illinois could be used to dry our co-product. These dryers are rotary steam tube dryers. Based on this assumption it was necessary to test dry our co-product to determine if it could be easily dried in this type of equipment. Quotes to conduct drying tests were sent to three manufacturers of rotary steam tube dryers, Davenport Machine, Swenson Process Equipment and Svedala Industries. Only two indicated interest, they were Davenport Machine and Svedala. *Davenport Machine was selected as they had the lowest cost for these tests and were willing to dry our co-product for the animal feed tests. Davenport setup a pilot plant size test system including a 300 lbs/hr rotary steam tube dryer, feed pumping system, dry recycle conveyors and steepwater mixing conveyors. They dried all our co-product for the animal feed tests, about 20,500 pounds. Due to time constraints for the testing of the dried material as animal feed, this material was dried without adding steepwater. Later tests were run using steepwater but not enough material was dried to use in any of the animal feed studies. Summary analysis of these drying tests are shown in Table 3.4.9.

While the full report from Davenport has not been received as yet, a few preliminary results have been included in this report. The Davenport pilot plant consisted of the following equipment:

Rotary steam tube dryer, 300 lbs/hr, 304ss
Feed pumping system, mixing tank and pump, cs
Steepwater drum pump, cs
Mixing/dryer feed screw conveyor, 304ss
Dry cake discharge screw conveyor, 304ss
Dry recycle screw conveyor. 304ss
Standard pulse air dust collector, cs
Dryer discharge blower, cs
Dryer discharge scrubber, FRP

Davenport used 105 psig saturated steam on the dryer which gave a vapor discharge temperature of 225°F and a dry cake discharge temperature of 160 to 3 65°F. The dry recycle rate was varied to give a feed composition of 23 to 27% moisture going to the dryer. Their evaporation rate was 1.41 Ibs of water per square foot of drying area. Davenport recommends a 1.47 rate for their commercial units, so scale up of this data will be easy. Steepwater, when added, was added at a rate that was consistent with its rate of generation in a typical wet mill (data supplied by Pekin indicated that steepwater is generated at a rate of 4.7 lbs DS per bushel, see Table 3.4.3 for the composition of typical steepwater).

The following are some observations of the drying tests:

1. Without steepwater the dry product was very dusty as noted by a very high rate of dust collection in the dust collector.
2. With steepwater dust collection was markedly reduced to almost none at all.
3. The dry product without steepwater was much lighter in color (dark tan in color) and the tendency to "ball" up was much less. The "balls" that did form were very soft.
4. With steepwater the dry product was noticeably darker in color (a dark brown) and produced a product that "balled" up into balls approximately 1/16 to 1/8 inches in diameter. Some however were as large as 1/4 inch and difficult to break up. A mill would have to be added to the design for co-product produced using steepwater (this was added to the flow sheets and capital costs).
5. Surprisingly, corrosion was a problem with the dust collector and blower. On inspection the dust collect showed signs of severe corrosion and the entire blower had to be replace during the tests due to being completely eaten up. The fan blade was particularly severely attacked. (The order of equipment was from the dryer the wet vapors first passed through the dust collector, then into the blower and then into the scrubber.)
6. Odors from the dust collector were noticeable and a scrubber was added, which helped and is the normal practice in all wet mills.
7. Analysis of the dust indicated that it had the same basic composition as the dry co-product. The analysis of the sludge in the scrubber indicated a relatively high level of protein (11.6%) and a high ash content (69.9%) which was mostly iron (48.2%). Interestingly Pekin observers indicated that the scrubber liquid from their plant had very high BOD and COD levels and had a major effect on the operation of their anaerobic treatment plant.
8. Some additional design information was obtain which included the following bulk densities:

   Wet cake - 65.5 lbs./cu.ft.

   Steepwater ▪ 74.0 lbs./cu.ft.

   Dry cake - 50.5 lbs./cu.ft.
9. From these tests it would appear that rotary steam tube drying may be too severe a drying method due to the free sugars being now present and their low tolerance for heat. A less severe form of drying should be studied, such as flash and spray drying.

Table 3.4.9

SAMPLE ANALYSIS FOR THE ANIMAL FEED STUDIES					
CATTLE, SWINE AND POULTRY					
	TYPICAL	SWAN CO-PRODUCT			
	CORN				WITH
ANALYSIS	GLUTEN	CATTLE	SWINE	POULTRY	STEEP
	FEED	FEED	FEED	FEED	WATER
DRY MATER, %	88	90.2	90.8	86.9	93.4
PROTEIN, %	21	21.7	21.5	23.1	25.5
CRUDE FIBER, %	10	12.9	14.2	13.1	13
ASH, %	7.8	11.7	11.9	8	10.1
CALORIES. CAL/LB	795	768	619	768	799
THREONINE, %	0.9	0.74	0.72	0.82	0.87
CYSTINE, %	0.5	0.39	0.41	0.43	0.4
VALINE, %	1.04	1.09	1.06	1.15	0.98
METHIONINE, %	0.5	0.32	0.32	0.37	0.31
ISOLEUCINE I, %	0.6	0.81	0.78	0.85	0.71
LEUCINE, %	1.9	2.56	2.55	2.82	2.38
PHENYLALANINE, %	0.8	0.97	0.95	1.03	0.93
HISTIDINE, %	017	0.49	0.48	0.45	0.46
LYSINE, %	0.6	0.17	0.17	0.19	0.22
ARGININE, %	1	0.17	0.15	0.25	0.34
TRYPTOPHAN, %	0.1	0.08	0.08	0.09	0.1
CALCIUM, %	0.2	0.04	0.04	0.11	0.05
COPPER, PPM	10	3.62	6.43	5.08	5.14
IRON, PPM	304	621	558	547	332
MAGNESIUM, %	0.42	0.08	0.08	0.15	0.23
MANGANESE. PPM	23.8	8.38	7.9	12.3	14
PHOSPHORUS, %	0.9	0.55	0.79	0.41	0.65
POTASSIUM, %	1.3	0.26	0.26	0.48	0.84
SODIUM, %	0.12	4.24	4.33	2.28	3.32
ZINC, PPM	88	87.6	79.7	85.8	100
SULFUR, %	0.16	1.04	1.06	0.58	0.96
SOURCE: REFERENCE #2 FOR TYPICAL CORN GLUTEN FEED					
HAZLETON ANALYSIS ON SEPARATE FEED SAMPLES					
	CATTLE - 09/12/96				
	SWINE - 09/12/96				
	POULTRY - 08/01/96				

Table 3.4.10

PROTEIN VALUE VS CORN PRICE		
CORN PRICE $ PER BU		PROTEIN VALUE $ PER LB
5.00		0.319
4.75		0.307
4.50		0.296
4.25		0.284
4.00		0.273
3.75		0.261
3.50		0.250
3.25		0.238
3.00		0.227
2.75		0.215
2.50		0.204
2.25		0.192
2.00		0.181

3.4.3.2 Pelletizing Results

A preliminary pelletizing study was conducted on our co-product by LCI Corporation. The LCI Granulation System for pelletizing granulated solids consisted of a Laboratory Batch Sigma Blade Kneader model KDHJ-20 and a Pellet Press model 14-175. The co-product they tested had a moisture level of 5.4% and a bulk density of (0.675 kg/l). It was mixed in the Kneader with 10.2% water giving a kneaded mass with a moisture of 15 %. This mixture was fed to the Pellet Press forming pellets of 3 mm diameter and 9 mm in length. This size press produced pellets at the rate of 105.9 kgihr. The pellets were produced at about 60°C with a final moisture content of 12.8%. These pellets looked very similar to the rice straw pellets produced earlier by LCI. LCI concluded that the SWAN co-product could be successfully pelletized in a commercial size unit but that further tests would be needed to properly size and price a commercial unit (see A-2.19 for the full report).

3.4.4 Animal Feed Studies

One of the major concerns of the Phase 3 tests was the value of the co-product produced. This product would compete with three other animal feeds (corn gluten feed, corn gluten meal and DDGS) in the feed market. The relative value of the SWAN co-product, on a dollar per ton basis, as well as its nutrimental value had to be determined. Because of the reduced volume of material, the SWAN co-product had to have nutrimental properties that were superior to its three competitors and thus bring a higher price per ton if it was to be competitive with the other three feeds. A plan and schedule was developed for the animal feed studies which is shown in Chart 3.4.1.

Chart 3.4.1

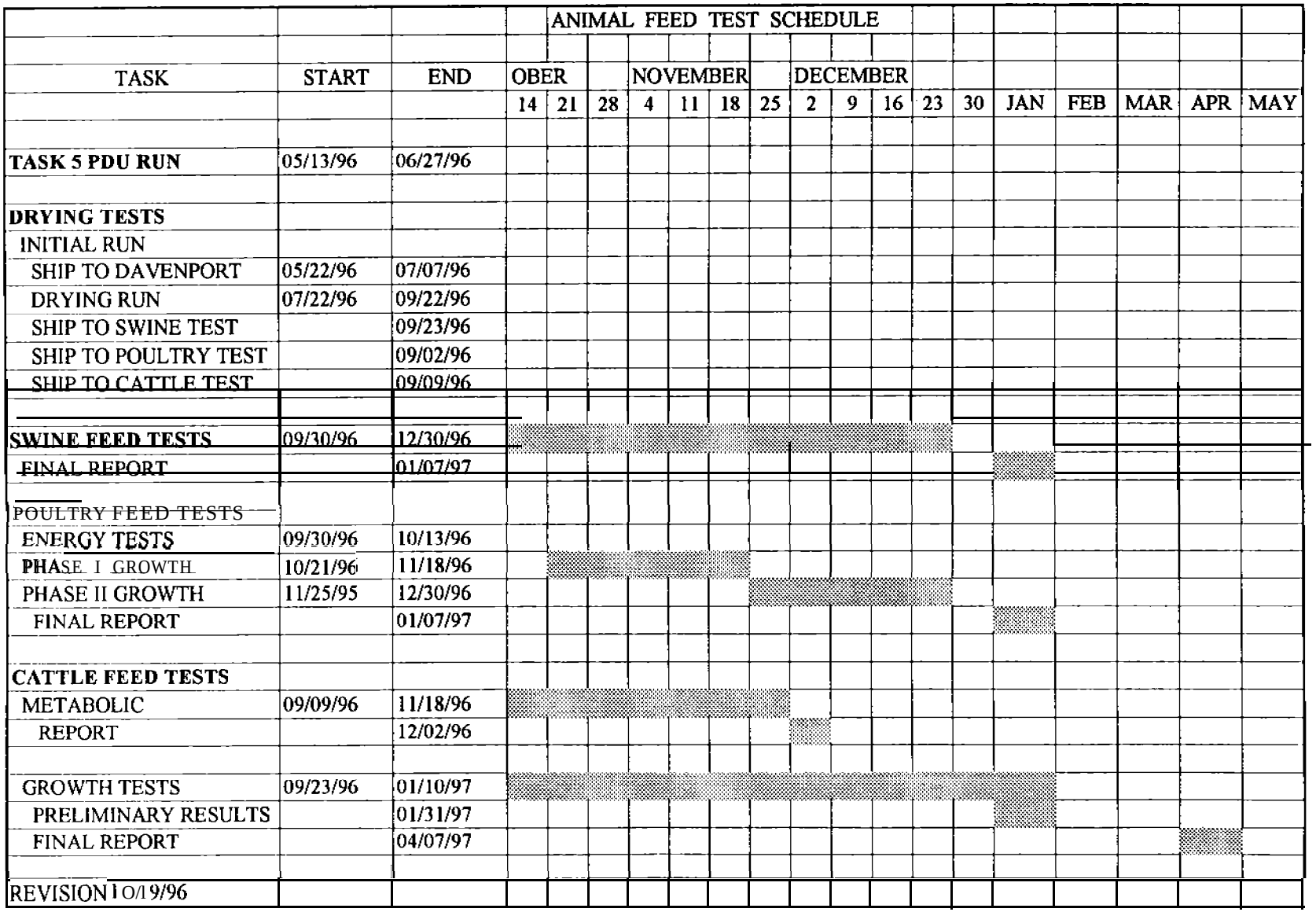

ANIMAL FEED TEST SCHEDULE
TASK
START
END
OBER
NOVEMBER
DECEMBER
14
21
28
4
11
18
25
2
9
16
23
30
JAN
FEB
MAR
APR
MAY
TASK 5 PDU RUN 05/13/96 06/27/96
DRYING TESTS
INITIAL RUN
SHIP TO DAVENPORT 05/22/96 07/07/96
DRYING RUN 07/22/96 09/22/96
SHIP TO SWINE TEST 09/23/96
SHIP TO POULTRY TEST 09/02/96
SHIP TO CATTLE TEST 09/09/96
SWINE FEED TESTS 09/30/96 12/30/96
FINAL REPORT 01/07/97
POULTRY FEED TESTS
ENERGY TESTS 09/30/96 10/13/96
PHASE I GROWTH 10/21/96 11/18/96
PHASE II GROWTH 11/25/95 12/30/96
FINAL REPORT 01/07/97
CATTLE FEED TESTS
METABOLIC 09/09/96 11/18/96
REPORT 12/02/96
GROWTH TESTS 09/23/96 01/10/97
PRELIMINARY RESULTS 01/31/97
FINAL REPORT 04/07/97
REVISION 10/19/96

Chart 3.4.1 (con't)

ANIMAL FEED TEST SCHEDULE

TASK	START	END	MAY			JUNE				JULY				AUGUST					SEPTEMBER					OC
			13	20	27	3	10	17	24	1	8	15	22	29	5	12	19	20	2	9	16	23	30	7
TASK 5 PDU RUN	05/13/96	06/27/96																						
DRYING TESTS																								
INITIAL RUN																								
SHIP TO DAVENPORT	05/22/96	07/07/96																						
DRYING RUN	07/22/96	09/22/96																						
SHIP TO SWINE TEST		09/23/96																						
SHIP TO POULTRY TEST		09/02/96																						
SHIP TO CATTLE TEST		09/09/96																						
SWINE FEED TESTS	09/30/96	12/30/96																						
FINAL REPORT		01/07/97																						
POULTRY FEED TESTS																								
ENERGY TESTS	09/30/96	10/13/96																						
PHASE I GROWTH	10/21/96	11/18/96																						
PHASE II GROWTH	11/25/95	12/30/96																						
FINAL REPORT		01/07/97																						
CATTLE FEED TESTS																								
METABOLIC	09/09/96	11/18/96																						
REPORT		12/02/96																						
GROWTH TESTS	09/23/96	01/10/97																						
PRELIMINARY RESULTS		01/31/97																						
FINAL REPORT		04/07/97																						
REVISION 10/19/96																								

3.4.4.1 Cattle Feed Study

The University of Illinois, Department of Animal Sciences submitted a test plan for $28,930 (see A-2.23) that was accepted by the Illinois Corn Growers Association (ICGA) to determine the protein and energy quality of the SWAN co-product for ruminants. These tests were under the direction of Dr. Dan B. Faulkner, Associate Professor and started in late September 1996. The analysis of the 6,300 pounds of co-product shipped to Dr. Faulkner is given in Table 3.4.9.

Specifically the objectives of this study were:

1. To evaluate the effects of limited feeding of the SWAN co-product. This feed will be compared to corn gluten feed fed to supply the same amount of fiber, corn gluten feed fed to supply the same amount of protein, and alfalfa hay fed to supply the same amount of fiber. These feeds will be fed with cracked corn to developing heifers and weight gains, and feed efficiency will be evaluated.
2. To evaluate these same diets in a steer metabolism study to determine digestibility, fiber digestibility, ruminal protein synthesis, undegraded ruminal protein and pH.

As of this writing these tests are underway and will be completed by the summer of 1997.

Cattle Feed Issues

A meeting was held in Bloomington, Illinois on March 26, 1996 to discuss the animal feed tests for cattle and swine. Issues dealing with cattle feed were presented by Dr. Faulkner and are summarized below:

1. Most of the corn gluten feed produced in the U.S. is used in ruminate diets and this would probably be the most likely use for our co-product.
2. There are two key proteins for ruminates and they are 1) degradable protein (DIP) and 2) undegradable protein (UIP). The UIP is very important for growth and will determine the market value of the SWAN co-product. At this time there are no analytical methods to measure the UIP of a given feed, so the only way to determine this is through feed studies.
3. The key components of any ruminate feed are, crude protein, acid detergent fiber, neutral detergent fiber, calcium, phosphorus, potassium and UIP. Amino acids distribution is not important. One of the major quality issues is consistency in the feed. This makes formulation easier.
4. Ammonium salts and high sulfates were not known problems with ruminates.
5. Color is not a problem in ruminate diets as the co-product would be blended with other ingredients up to a level of 15 % in the total diet.
6. Crude fiber is a measure of the total amount of fiber in the feed. The SWAN co-product had a high level of crude fiber (not unlike hay) and thus would not be a problem for ruminates.
7. The SWAN co-product will be rated against normal corn gluten feed and alfalfa hay and priced against soybean meal which in July of 1996 was selling for $260 per ton.

The cattle feed studies will include two tests, the Heifer Performance Trial and the Digestion Trial.

Heifer Performance Trial

The purpose of this trial is to compare the SWAN co-product to corn gluten feed in supplying fiber and protein to cattle under a limited feeding schedule. Heifers will be randomly allocated to pens and full weights taken to establish beginning weights. Weights will be taken every 28 days during the test. Three replications of 12 pens with eight heifers per pen will be run for four diets. These diets are: 1) a 30% SWAN co-product and

70% cracked corn , 2) a 30% corn gluten feed and 70% cracked corn, 3) a 45% corn gluten feed and 55% cracked cam and 4) a 30% alfalfa hay and cracked corn. Feed is limited to attain a daily gain of about 1 kg. Comparisons of diets 1 and 2 will evaluate the protein value of the two feeds, comparison of diets 1 and 3 will evaluate the fiber value of the two feeds and diets 1 and 4 will evaluate the SWAN co-product fiber with alfalfa hay.

Digestion Trial

This trial will measure total tract digestibility, fiber digestibility, fluid dilution rate (ruminal protein synthesis) and particulate passage rate (undegraded ruminal protein) and pH on four Angus steers utilizing the four diets used in the Heifer Performance Trial in a 4 x 4 Latin Square design and analyzed according to GLM procedures.

3.4.4.2 Swine Feed Study

Western Illinois University, Agriculture Department submitted a test plan for $22,230 (see A-2.22) that was accepted and funded by the ICGA to determine if the SWAN co-product can be economically used to formulate swine finishing rations. This test will be under the direction of Dr. John Carlson, Professor, and were started in early November and will be completed in 3 to 4 months. The analysis of the 7,200 pounds of co-product shipped to Dr. Carlson is given in Table 3.4.9.

Swine Feeding Issues

Dr. Carlson commented on swine feeding issues at the March 26 meeting in Bloomington. They are summarized here.

1. Lysine and fiber are important in swine diets. High lysine and low fiber content are preferred. He thought that we may have to supplement our co-product with synthetic lysine to the level of 5 to 6 %.
2. The SWAN co-product would have to be competitive with soybean meal.
3. Critical analysis for swine diets are amino acids such as lysine, tryptophan, methionine and cystine; minerals calcium, phosphorus, chlorine, sodium and sulfur; crude protein and crude fiber.
4. High sulfur levels could be toxic to swine (see section 3.4.5.3).
5. Neutralizing with lime (forming calcium sulfate) is preferred over sodium sulfate (neutralizing with sodium hydroxide). Calcium is a positive mineral.
6. Swine diets prefer less fiber in that fiber fills the pig and keeps him from eating more of the diet that will add weight.
7. Our co-product would be used in diets for pigs after they have reached a weight of 100 pounds. This is due to the fact that young pigs do not perform well with diets high in fiber.
8. He expressed no concern with ammonium salts, arabinose levels and color.
9. Hogs are also being sold on a carcass merit basis, which is normally based on the backfat of the pig.

Finishing Phase Feed Study

One hundred and forty pigs (both females and males) will be selected after an initial feeding period to bring them up to approximately 100 pounds in weight. Groups of 28 pigs will be feed one of the following five diets; (1) a corn - soybean meal diet, (2) a diet similar to #1, except with 10% corn gluten feed, (3) a diet similar to #1, except with 30% corn gluten feed, (4) a diet similar to #1, except 10% SWAN co-product and (5) a diet similar to #1, except with 30% SWAN co-product. This allows for the low (10%) and high (30%) diets containing SWAN co-product to be compared with its competitor corn gluten feed as well as with the standard corn-soybean meal diet.

Each treatment group will be assigned to one of four pens for replication. The tests will be carried out under commercial conditions with each pen holding 7 pigs in a pen area of 6 feet by 16 feet equipped with a feeder. The weight of feed used to each feeder is recorded to determine feed consumption and the subsequent feed efficiency of weight gain on a per pig basis. These three factors (feed consumption, feed efficiency and weight gain per pig) will be analyzed at 42 days (approximate mid-point) into the test and also at the conclusion when the pigs reach a weight of 240 pounds. The pigs will be slaughtered and the backfat recorded so that this trait may also be analyzed for diet differences. If the SWAN co-product proves beneficial, future trials might be initiated to investigate the its effects on younger pigs.

3.4.4.3 Poultry Feed Study

The University of Georgia, Department of Poultry Science Extension, submitted a test plan for $16,200 (see A-2.20) that was accepted and funded by the ICGA to determine the nutritive qualities of the SWAN co-product in normal broiler (chicken) diets and to determine the economic value of the co-product. These tests were under the direction of Dr. Nick Dale and were started on September 17, 1996. The analysis of the 1,000 pounds of co-product shipped to Dr. Dale is given in Table 3.4.9

Poultry Feeding Issues

A meeting was held with Dr. Dale and Phil Shane of the ICGA in Athens, Georgia on April 3, 1996. Dr. Dale's comments on his proposed feed studies are summarized below;

1. There are two major poultry markets, broilers and layers (there is a third for turkeys which Dr. Dale felt could be explored later if the chicken tests were successful). Each one has a different dietary need. Our most likely market would be broilers. Broilers require more energy than layers while young broilers need more protein than older birds.
2. Typical poultry diets include the following ingredients:

Corn	55 to 60% of the total diet
Soybean meal	30 to 35%
Animal protein	5 to 8%
Others	4 to 5%

The others category includes such things as fat, lime stone, corn gluten meal and stale bakery products for energy. The SWAN co-product would be used to replace the soybean meal up to 15% of the total diet.

3. Poultry feeds need at least 18% protein. The major important amino acids are lysine, methionine and cystine. Minerals and fiber are not important in poultry diets.
4. Co-product form and color are not issues as the diet is formulated and then pelletized so the chickens can pick it up more easily. Sodium and phosphates need to be in the right ratios and can be adjusted with limestone or salt.
5. He suggested ways we could enhance our co-product if the tests proved unsuccessful. Energy could be increased by adding lecithin or soap stock from soybeans and lysine could also be added.

The proposed poultry feeding tests would be done in three part: 1) metabolic; 2) starter diets; and 3) market age tests.

Metabolic Test

A small quantity of SWAN co-product would be fed for a given length of time (normally 2 days) and the energy valve (in cal/lb) determined. The results of this test help determine the suitability of the co-product as either a broiler feed or layer feed. This test was completed on October 4, 1996 and gave a value of 1010 cal/lb. This indicated that the SWAN co-product has an intermediate energy value (better than wheat millings but not as good as soy meal for example).

Starter Diet Tests

The purpose of these tests is to confirm the Metabolic test and to set nutrient parameters for practical growing poultry diets. These tests would be conducted under commercial conditions (using feeding pens with up to 50 birds per pen) using the following diets, 1) base diet, 2) 5% SWAN co-product, 3) 10% SWAN co-product and 4) 15% SWAN co-product. The four diets would each be fed to 64 broiler chicks (8 pens with 8 chicks each) from 1 to 18 days of age. The rapid growth of the chicks during this test makes the chicks sensitive to changes in the nutrient content of the diet. These tests will be started in late October and should be completed by the end of November.

Market Age Tests

The final poultry test would be conducted if the starter diet tests prove successful. In these tests, the birds are started on the starter diet and grown to full size (about 42 days) using a finisher diet again under commercial practices. The test design would be very similar to the starter diet test, except the eight pens would contain 50 birds instead of eight, as in the starter diet test. The starter diet would be used from 1 to 21 days of age and the finisher diet from 22 to 42 days of age. These tests have two objectives. The first is to measure body weight gain and feed conversion (pounds of feed required to produce one pound of weight gain) and second to check for unexpected problems, in our case the effects of a high level of sulfates.

3.4.4.4 • Swan Co-Product Value Determination

A significant factor in the price per gallon of ethanol produced by the SWAN Biomass process is the credit received from the co-product. For corn fiber, the most logical way is to base this price ($ per ton of co-product) on the pounds of protein fed to the process. The protein is not effected by the process, it simply passes unchanged through the process and out with the co-product (some soluble proteins maybe lost in the waste water steams but this has not been quantified and is probably not significant). The less co-product produced (higher conversions) the higher the percent protein and thus the higher the value of the co-product. This method does not take into account the potential increase in value of the co-product thorough higher energy values or other value added properties, but these can not be measured until the animal feed tests are completed. At this point the only way to value the SWAN co-product is the protein value method.

To determine the value of a pound of protein, the current value of a bushel of corn and the current value of the various feed co-products had to be analyzed and a correlation developed between corn prices and the value of a pound of protein. The co-products studied were, (1) corn gluten feed (CGF), (2) corn gluten meal (CGM) and (3) DDGS. The standard values (from reference 2) used for this analysis were:

Percent Protein in Corn	10.29
Percent Water in Corn	15.50
Percent Protein in DDGS	29.00
Percent Protein in CGF	22.00
Percent Protein in CGM	60.00
Percent Water in all Co-Products	10.00

Pounds per Bushel of Corn **56.00**

Using data from December, 1991 to April, 1996, that was provided by the Illinois Corn Growers Association and data from reference 4, a simple linear regression correlation, using all the data for all three co-products, was developed. This correlation is:

X = 0.04605 (Y) + 0.08867

where

X = The value of protein, $/lb.

Y = Corn price, $/bushel

The correlation coefficient was 0.588, which represents only a fair correlation. Shifting the data for a three month lag time did not increase the correlation coefficient. No attempt was made to look at any curvilinear relationships. Table 10 gives protein values vs. corn prices based on this correlation. From this table, the protein value will range from $0.319/lb for corn at $5.00 per bushel to $0.181/lb for corn at $2.00 per bushel. For the Pekin case this equates to a price per ton for the co-product of between $192 and $109.

3.4.4.5 Co-Product Sulfate Levels

Early in the undertaking of Phase 3, discussions were held with A.E. Staley, one of the largest corn wet millers in the U.S. Some of these discussions related to animal feeds and their quality. One specific comment was made that corn gluten feed or any animal feed that would replace it could not have a sulfate level greater than 0.5% (equivalent sulfur level of 0.167%). This was a major concern for the SWAN process since we used large quantities of sulfuric acid which are neutralized in the process to either ammonium, calcium or sodium sulfates, depending on which base is used. These sulfates, which are highly water soluble, would be dissolved in the thin stillage and would be carried over to the co-product with any liquid in the wet cake from the decanter centrifuges. As discussed in section 3.4.3.2.1 we became very concerned about the sulfate level since our wet cake was only 22 to 23% solids. This meant considerable amounts of sulfates would be in our co-product. As expected, drying tests indicated that as the water was removed the sulfates deposited on the co-product producing a co-product with a sulfate level of 1.7% to 3.2%. A study was then undertaken to determine if the sulfate levels could be reduced or if in fact this level of sulfates was a problem.

Our first thoughts were to substitute phosphoric acid for sulfuric acid; however this proved technically impossible because the disassociation constant for phosphoric acid was too low and since all of our past data was with sulfuric acid it was decided to stay with sulfuric. Batch washing of the solids before centrifugation was considered and tried on the laboratory scale. Even with small quantities of wash water, which did reduce the sulfate level, too many of the solids were lost and this was dropped as a viable option.

A study was then undertaken to determine if sulfates were indeed toxic to cattle, swine and poultry at the 0.5% or above level. Discussions with Doctors Faulkner, Carlson and Dale (the professors who were running our animal feed tests) indicated that only Dr. Carlson was aware of any work done in this area (see section 3.4.5.1.2). After considerable help from Faulkner, Carlson and Dale the following data was collected on the toxicity of sulfates and sulfur to cattle, swine and poultry:

Beef Cattle	0.4% as sulfur in the total diet
Dairy Cattle	0.26% as sulfur in the total diet
Poultry	8,100 to 14,000 ppm as sulfates in the total diet (equivalent to 0.27% to 0.47% sulfur)

Swine	No information found

All this data was obtained from the National Research Council (1980). This data indicated that the sulfates or sulfur levels would always be measured against the total diet and not the single ingredient. To complete any evaluation of the effects of the SWAN co-product's sulfate level on the final diets, the sulfur or sulfates level of the other potential ingredients had to be considered. Again with the help of Doctors Faulkner, Carlson and Dale (again the source was the National Research Council) the following sulfur levels were determined for the major components of most cattle, swine and poultry diets.

Corn	0.11% sulfur
Soybean meal	0.44% sulfur
Corn gluten meal	0.29% sulfur
Corn gluten feed	0.16% sulfur
DDGS	0.30% sulfur

Using the most stringent sulfur level for comparison, that for dairy cattle of 0.26% in the total diet, and a diet of (see section 3.4.5.1.1) 30% of SWAN co-product and 70% of cracked corn and a sulfur level of 0.89% (an average of the three analysis in Table 5) in the SWAN co-product, the total diet would contain 0.344% sulfur. In a more normal diet of 15% SWAN co-product the sulfur level would be 0.228%. These are just below the values shown above. Based on this information no attempts at sulfate removal were made and Doctors Faulkner, Carlson and Dale felt the animal feeding tests should proceed. If on a commercial scale we can improve the separation at the centrifuge to 30% solids these two sulfur values would drop to 0.272% and 0.192% respectfully.

3.4.5 Conclusions

1. The SWAN process will generate the following quantities of co-product with a feed rate of 750 dry tons per day:

Without steepwater	449 dry tons per day
With steepwater	707 dry tons per day

2. The SWAN co-product will have the following protein levels:

Without steepwater	18 to 19%
With steepwater	25 to 27%

3. The protein level used in the spreadsheet model runs was too low at 7.5%. This should have been at the 10 to 11% level.
4. With the SWAN process the current PDU decanter configuration would produce wet cake solids at 22 to 24% and have solids recoveries of approximately 60%.
5. The decanter settings that gave the best results were:

Feed rate	2.0 gpm
Back drive speed	6.5
Pond depth	4.5

6. Komline-Sanderson tests indicated that the use of a rotary vacuum filter to separate the SWAN whole stillage is not practical.
7. Alfa Lava1 centrifuge tests (confirmed by Bird Machine tests) indicate that the maximum solids in the wet cake will be 33.870 but that normal levels will be below 30%. Maximum solids recovery can be as high as 94.9%.

8. The Alfa Lava1 BD disc conveyor will give the best solids recovery and is recommended for the commercial size decanter.
9. Centrifuge tests indicated that there is a trade off between high percent solids in the wet cake and solids recovery (high solids in the wet cake corresponds to low solids recovery).
10. Evaporation of the thin stillage from corn fiber/screenings to recover the soluble proteins is not economical.
11. The SWAN co-product can be dried in a rotary steam tube dryer, however there are some indications that this type of dryer may down grade the product by darkening it and possibly reducing its nutritional value.
12. Milling of the SWAN co-product will be necessary when using steepwater.
13. Due to the corrosiveness of the co-product, the rotary dryer will have to be made of 304 SS (at least the tubes and a portion of the shell).
14. The SWAN co-product was pelletized successfully in a LCI pellet mill.
15. The value of the protein in the SWAN co-product can be related to the price of corn using the equation in section 3.4.5.2.
16. The SWAN co-product using the current process will produce a co-product with sulfates levels of between 1.7 and 3.2%. In total feed diets the sulfur levels will range between 0.228 and 0.344% which is comparable to the most restrictive level known (dairy cattle at 0.26%).

3.4.6 References

1. Hutjens, M.F., Hollis,G.R., Berger,L.L., Parsons,C.M., Easter,R.A., Weigel,J.C., Bidner,S.G., (1988). Corn Gluten Feed. The Future of Feeding. Bloomington, IL; Illinois Corn Growers Association.

2. Dale, N., (1994). Feedstuffs Ingredient Analysis Table. Athens, GA.; University of Georgia.

3. Watson, S.A., Ramstad, P.E. (ed), (1994). Corn: Chemistry and Technology. St. Paul, MN; American Association of Cereal Chemists, Inc.

4. OXY-FUEL NEWS, January 9, 1995 to April 1, 1996.

3.5 PDU Model

Predictions made by the final form of the kinetic model (see Section 2.2) are compared to measured data from Tasks 3 and 5. The model's predictions are not compared to Task 4 data, because HMF and furfural were not accurately measured.

Figure 3.5.1 shows modeled (lines) and measured (points) concentrations of glucose, xylose, and ethanol in the 7000 L batch fermentation during Task 3. Figure 3.5.2 shows concentrations of glucose, cellulose, and cellobiose. At 18 hours, the model predicted glucose concentrations lower than measured. After 18 hours, low monomeric glucose concentrations are detected by HPLC and YSI measurements show little or no glucose present. The HPLC glucose levels are probably elevated due to baseline problems with corn fiber chromatography that typically show up with Biorad columns, so the predicted glucose concentration is probably close to the actual concentration. The modeled xylose and ethanol concentrations (after full conversion of glucose) are close to measured concentrations. Cellulose conversion appears to be modeled reasonably well, because the measured cellulose concentration at 108 h is close to the modeled concentration.

However, measured cellobiose concentration is higher than predicted at many time points, but this difference is again due to baseline measurement problems.

The Task 3 HMF measurements are near the detection limit of the NREL HPLC system, so the error could be significant. To see the effects of a possibly higher HMF concentration, Task 3 was modeled a second time with an initial HMF concentration of 0.5 g/L, as opposed to the measured concentration (0.33 g/L). The results of the second modeling are shown in Figures 3.5.3 and 3.5.4. The predicted glucose and ethanol concentrations at the time points before 18 h are much closer to measured.

Figures 3.5.5 and 3.5.6 show the measured and predicted concentrations of ethanol, xylose, and cellulose in each fermenter during Task 5's first and second mass balance points, respectively. Oligomeric glucose and xylose were converted to ethanol during the second point. These amounts were entered into the model as additional monomeric sugars, because conversion of oligomeric sugars has not been modeled. The measured xylose concentrations in the first and second 9000-L fermenters is lower than predicted in both cases. The discrepancy was also seen in the chemostat and may be caused by extra utilization of xylose during and after glucose utilization. The cellulose concentration in the third 9000-L fermenter is lower than predicted in both cases. However, changes in pretreatment conditions since the constants were first determined, different mixing properties, or running in continuous mode, could increase cellulose conversion. If the predicted cellulose conversion were closer to the measured conversion, the ethanol concentration would be closer to the measured value. The predicted ethanol concentration in third fermenter is 5.5% lower than the measured concentration for both mass balance points.

Batch fermentations can be predicted well with the SSCF kinetic model. The only serious error seems to be the utilization of xylose for 2448 hours after glucose is utilized. Continuous fermentations may be modeled well if the cell reduction expression holds true, but more continuous work will be necessary to make that determination.

Figure 3.5.1: Task 3 Batch Fermentation

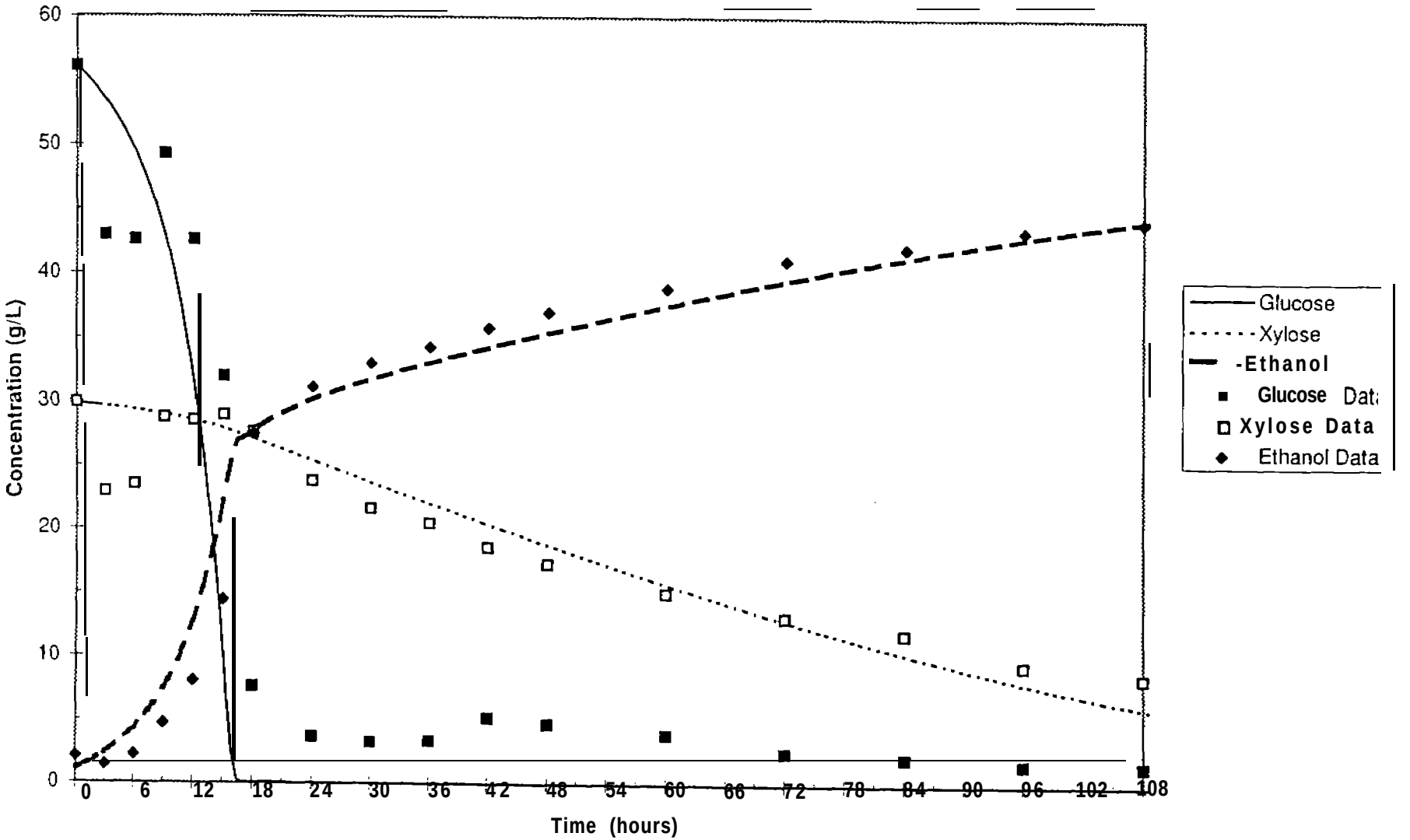


Figure 3.5.2: Task 3 Batch Fermentation

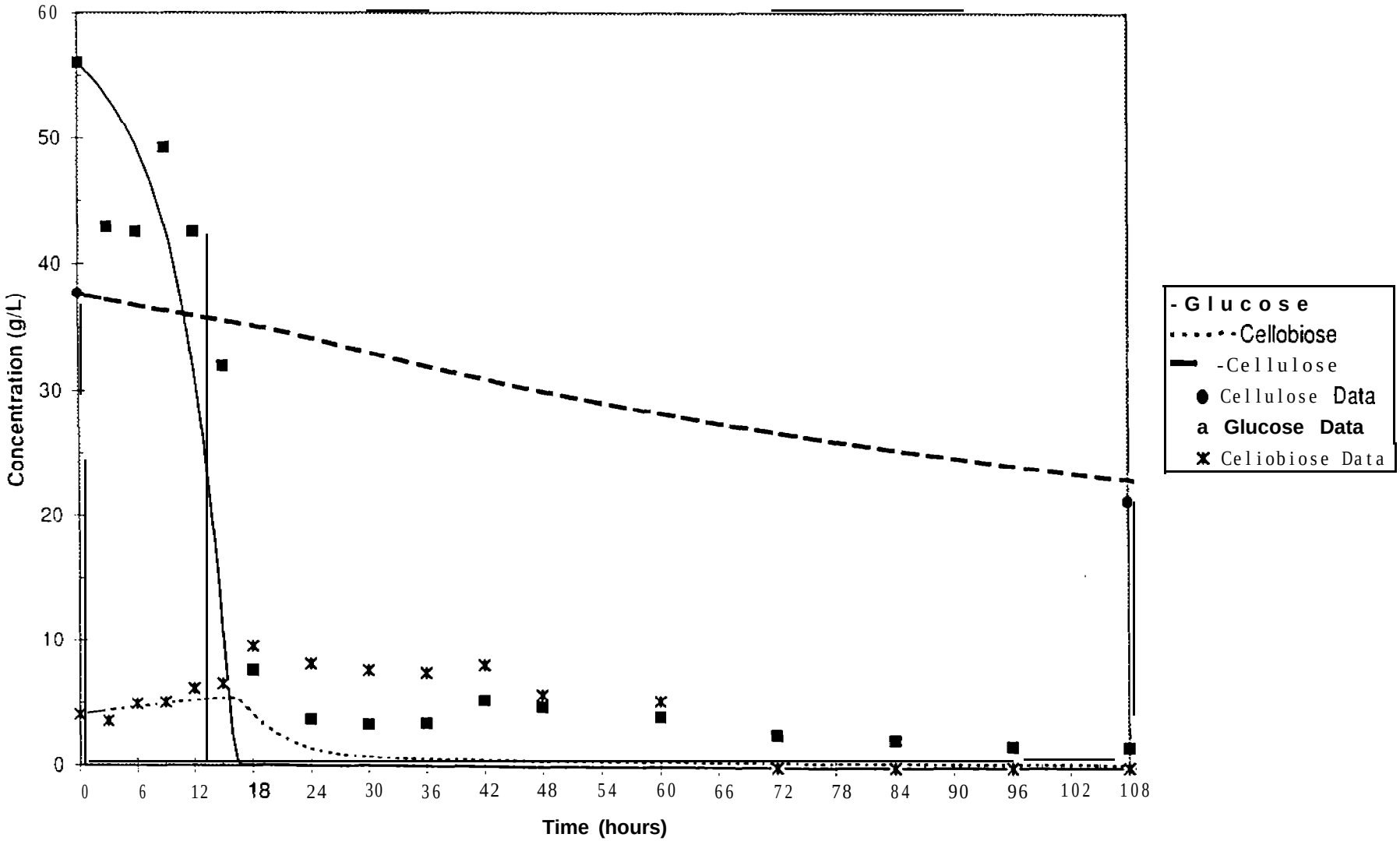


Figure 3.53: Task 3 with Modified HMF Concentration

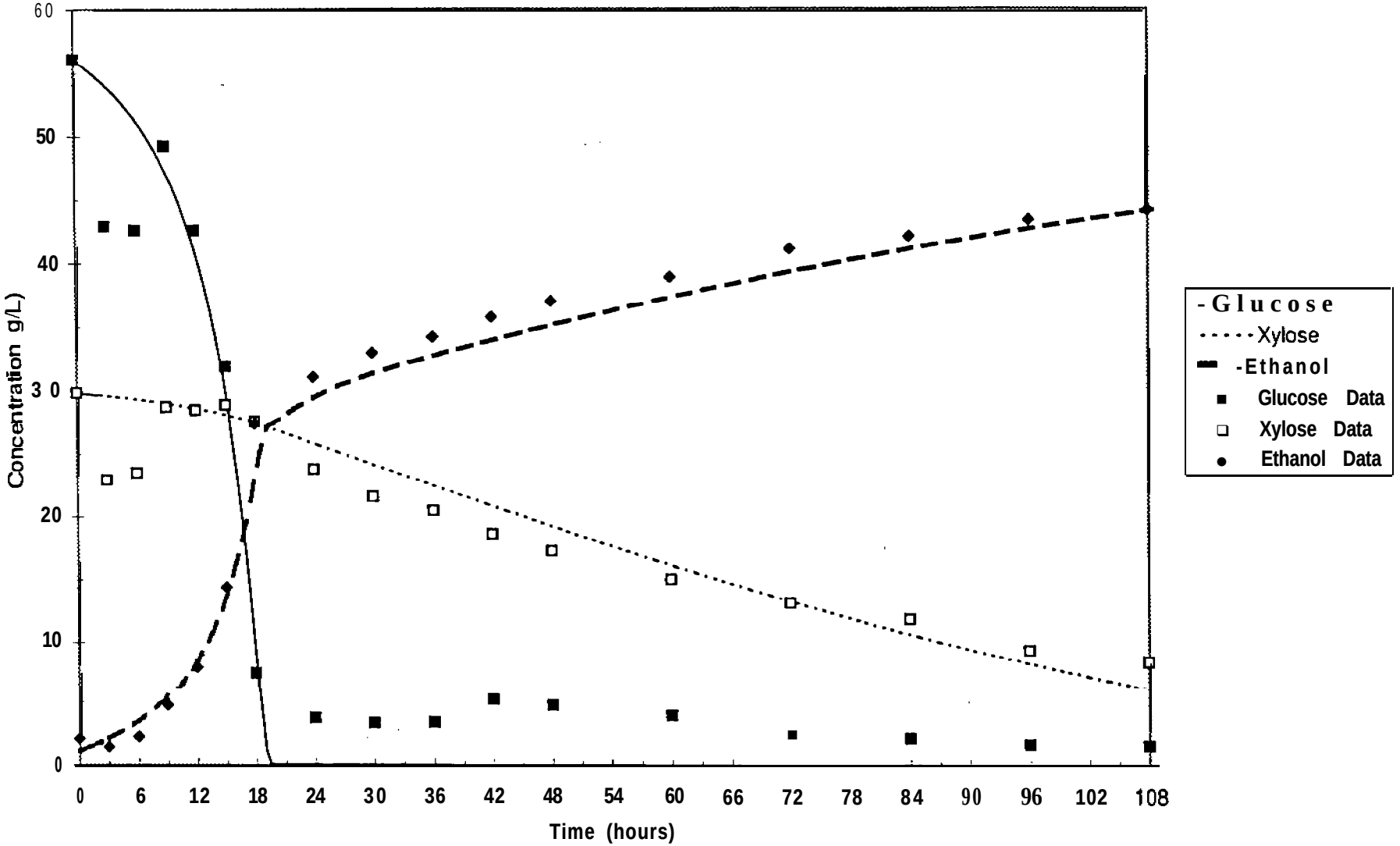


Figure 3.54: Task 3 with Modified HMF Concentration

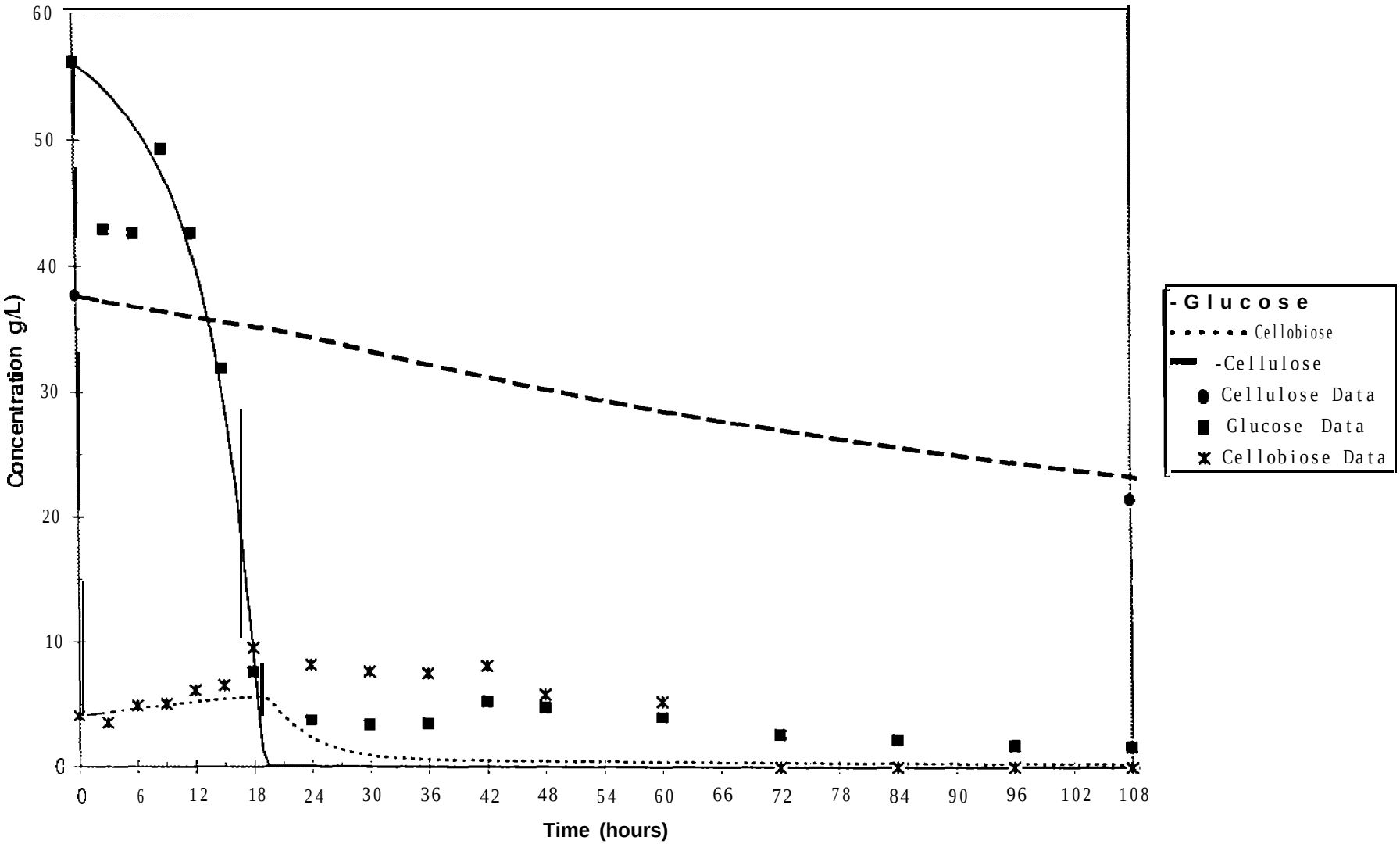


Figure 3.5.5. Concentrations at the First Task 5 Mass Balance Point

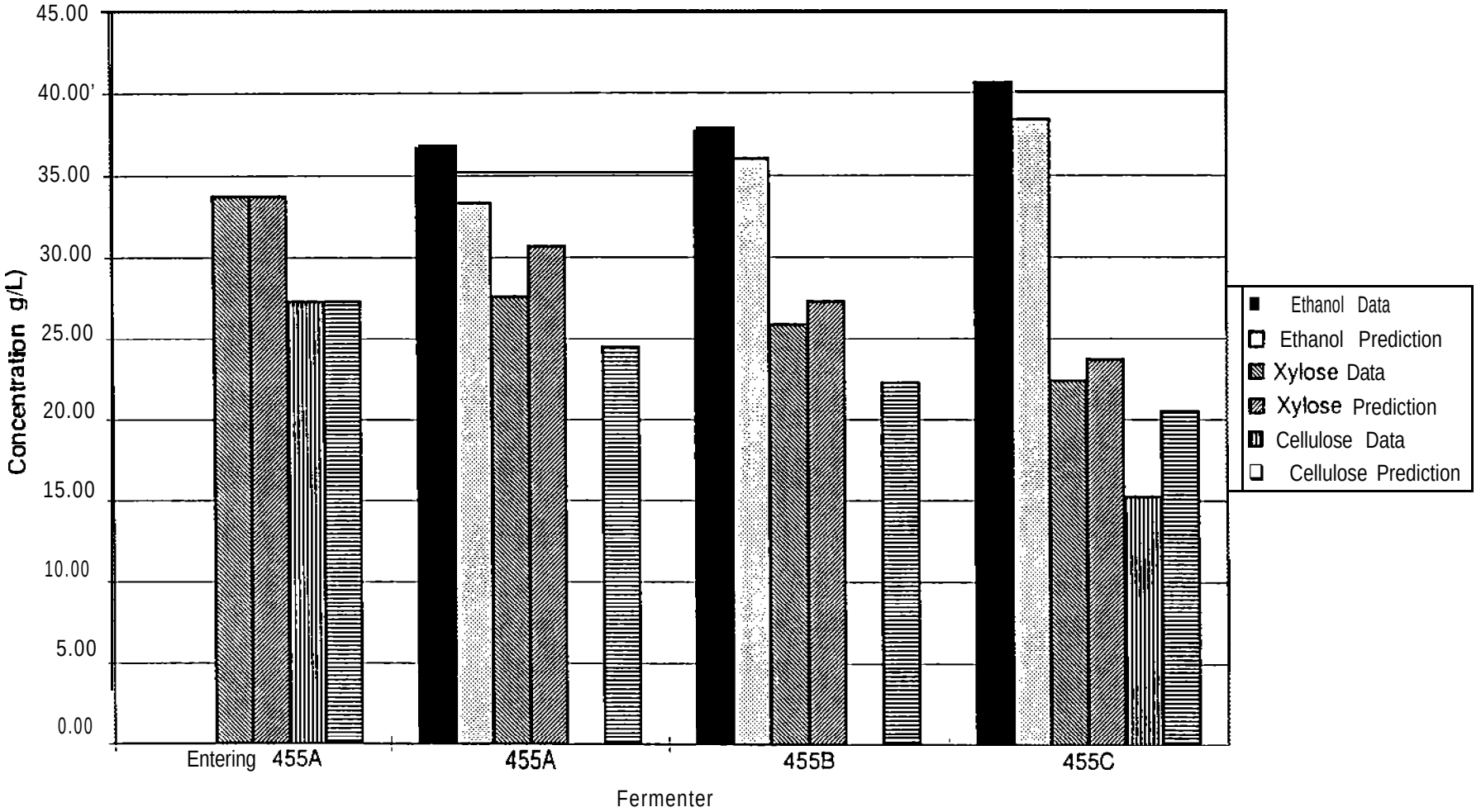
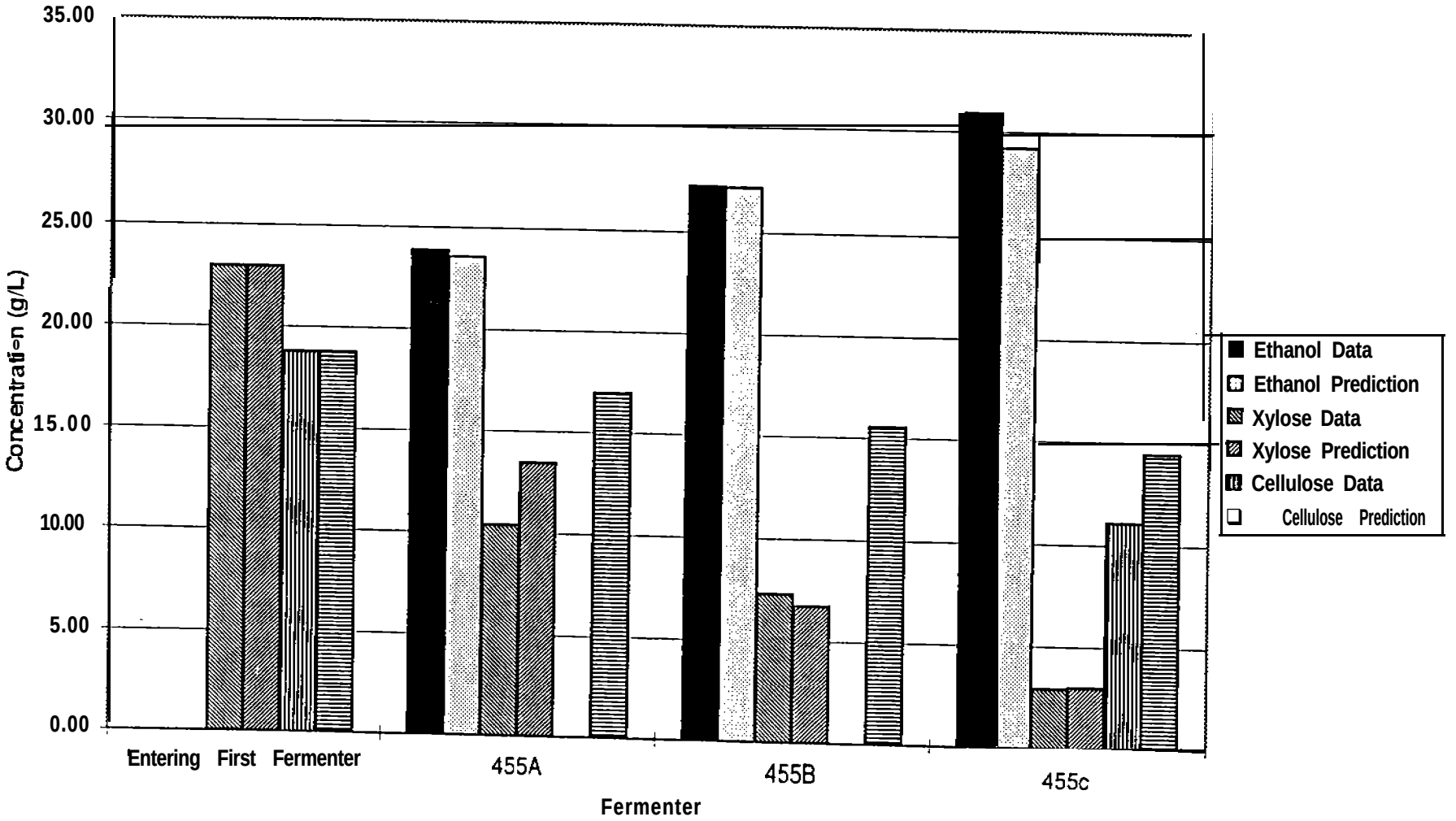


Figure 3.56. Concentrations at the Second Task 5 Mass Balance Point



3.6 Overall PDU Performance

The composition and standard deviations of a corn fiber blend are shown in Table 6.3.1. These values are averages of all composition data generated during Task 4 and 5 and were used as the feedstock composition for economic analysis work. Some measurements performed by an outside laboratory on contract with NREL were not used. Starch measurements are the most difficult to make and were the most variable ranging from 17.5%-39.0% with an average of 27.2% and a standard deviation of 6.8%. Cellulose was the difference between total glucose and starch and has a high standard deviation because of the large uncertainty in the starch measurement.

Table 3.6.1. Average Composition of the Corn Fiber Blend

Component	Dry Weight Composition (%)	Standard Deviation (%
Starch	27.2	6.8
Cellulose	12.2	7.2
Galactan	3.5	0.5
Xylan	16.4	1.7
Arabinan	10.6	0.9
Lignin	3.4	OS
Acid Soluble Lignin	4.0	0.4
Ash	0.7	0.1
Protein	7.5	1.3
Acetate	2.5	ND
Other'	12.0	3.5

Average moisture content was 54.3% (0.9% standard deviation)
ND -Not determined
'Other is 100 minus all other components

Table 3.6.2 summarizes fermentation performance information and observations for all CRADA PDU runs. The runs with 1400 using SSF were useful for mechanical check out of plant operation, but did not provide relevant performance information. Experience was gained on continuous operation of both the APR and fermentation equipment during Task 2. However, because of the poor pretreatment performance (see discussion and figures in section 6.2), no information useful for economic evaluation of the process was generated on either pretreatment or fermentation. Information was gained during operation of the plant in Task 3 through Task 5 because of the use of the recombinant yeast and better pretreatment performance.

The batch fermentations performed at the bench scale and in PDU fermenters during Task 3 showed no significant performance differences and proved that batch bench data mimics PDU data (Appendix A-2, Task 3 Run Report). Similar results were also obtained during bench-scale continuous fermentations and PDU runs, as discussed in section 6.3. Although, there was uncertainty in comparing continuous bench data and **PDU** results because of variable pretreatment performance, which produced varying levels of acetic acid. Terms expressing organic acid inhibition of xylose fermentation and inhibition by furfural and HMF were added to the kinetic model (section 2.2) and proved helpful in predicting PDU fermentation performance (section 3.5).

Tasks 4 and 5 provided performance information on continuous SSCF using LNHST2. A comparison between the two runs at the 25% solids levels shows better performance during Task 5 (see Table 6.3.1). The increased performance was due to a more severe pretreatment that produced more sugars (particularly monomeric xylose), so more sugars were converted and ethanol produced even though inhibitor levels were higher. It was noted that changes in pretreatment severity, particularly the decrease in severity that occurred throughout Tasks 4 and 5 did have an effect on fermentation' performance.

The most complete (and with a higher degree of confidence) mass balance data was taken at two points during Task 5 and more details on conversions and yields are shown in Table 6.3.3. These results are the best data obtained during any PDU run and show the current performance of the process at two solids levels. Comparing results shows the importance of inhibitor concentration, since inhibitor concentration is lower at the lower solids level. There is a significant increase in xylose conversion at the lower solids level and a subsequent increase in ethanol process yield from 47% to 55%. However, the low process yields are due to a large fraction of sugars in the feedstock not being converted to ethanol. Of the sugars being converted (glucose, galactose, and xylose), 80%85% are converted to ethanol and the rest to by-products (cell mass, glycerol, xylitol, etc.).

Table 3.6.3. Conversion (%) and Yield (%) Information at the Two Mass Balance Points From Task 5

	First Point 25 % Solids Concentration Ethanol Conc. 37.4 g/L	Second Point 15 % Solids Concentration Ethanol Conc. 29.6 g/L
Pretreatment		
Fraction Cellulose Hydrolyzed	16.7	4.4
Starch to Total Soluble Glucose	99.1	99.8
Acetate to Acetic Acid	63.7	19.7
Xylan to Total Soluble Xylose	85.0	95.7
Xylan to Monomeric Xylose	67.2	47.9
Arabinan to Total Soluble Arabinose	76.1	88.2
Arabinan to Monomeric Arabinose	62.3	57.5
Glucose to HMF	0.6	0.2
Xylose to Furfural	2.8	1.8
Fermentation		
Fraction Cellulose Hydrolyzed	44.3	41.3
Total Soluble C6[1] to Cell Mass	2.9	4.1
Total Soluble Glucose to Glycerol	7.5	9.9
Monomeric Xylose to Xylitol	7.5	26.3
Monomeric Xylose to Ethanol	26.2	53.2
Total Soluble C6 to Ethanol	67.1	79.5
Total Process Yield	46.9	55.1
Total Metabolic Yield	84.6	82.2

[1]C6 is glucose and galactose

The form and percentage of sugars entering and leaving SSCF and conversions at the two mass balance points of Task 5 are shown in Table 3.6.4. If the amount of sugar entering fermentation is low (e.g., starch), the conversions are suspect due to experimental error (e.g., negative values) and can be ignored. Cellulose and

oligomeric and monomeric glucose and xylose are the main sugars entering fermentation. The only sugar completely converted was monomeric glucose. Converting additional oligomeric glucose and both forms of xylose would presumably improve process economics. If all of the glucose, galactose, and xylose were converted to ethanol, the process yield would improve to 72% (assuming 80% conversion of the sugars to ethanol) and 77.5% if the rest of the cellulose was also converted. Cellulose may not be economically recoverable **because** of the high cost of purchased enzyme. The economics of cellulose conversion could change if cellulase enzyme is made on-site instead of purchased, but no data is available to determine cellulase production cost and performance.

Table 3.64. Form and Percentage of Sugars Entering, Leaving and Converted During SSCF (Task 5

	First Point (25% solids)			Second Point (15% solids)		
	% of Total		% Converted	% of Total		% Converted
	In	out		In	out	
Starch	0.4	1.1	-14.3	0.5	1.6	0.0
Cellulose	16.4	20.6	44.2	17.5	31.7	41.9
Galactan	0.4	0.7	28.6	0.1	0.5	-200.0
Xylan	1.6	1.2	66.7	1.3	2.3	45.8
Oligomeric Glucose	13.4	24.6	18.4	22.7	29.7	58.0
Monomeric Glucose	38.3	0.8	99.1	26.6	0.7	99.2
Oligomeric Galactose	0.2	2.2	-300.0	2.0	4.5	27.8
Monomeric Galactose	3.5	6.1	22.4	2.5	3.6	53.3
Oligomeric Xylose	5.4	12.4	-2.2	13.4	17.0	59.5
Monomeric Xylose	20.3	30.4	33.6	13.4	8.5	79.7

The increased xylose conversion at 15% solids during Task 5 was due in part to a conversion of approximately half of the oligomeric xylose to monomeric form in the fermenters and then subsequent conversion to ethanol. Conversion of oligomeric xylose was not seen in any other runs. Possibly because the lower xylose concentration achieved at this point in the run may be low enough to favor chemical or enzymatic hydrolysis of the oligomeric xylose or a shift in equilibrium. But, the oligomeric xylose concentration was also higher at this point in the run **because** of lower pretreatment severity. The monomeric xylose to total soluble xylose ratio was near 80% during the early part of the run, but dropped to 50% at the second mass balance point.

Overall, organism performance is affected by pretreatment performance (as reflected by inhibitor and sugar levels) and also by high ethanol levels as shown by bench scale testing. The kinetic model and better maintenance of high pretreatment performance will be necessary to economically optimize the process. Adapting or improving yeast performance (i.e., achieving better xylose conversion) at higher solids concentration would improve economics of the process.

Table 3.6.2. Fermentation Performance Summary for All PDU CRADA Runs (All data from mass balance points)

Run	Mode	Organism/ Process	Effective Solids Conc.[1] (%)	Ethanol Conc. (g/L)	Glucose Conv.[2] (%)	Xylose Conv.[3] (%)	Ethanol Process Yield[4] (%)	Comments/Lessons Learned
P950206CF	Batch	1400/ SSF	10	18	NA	NA	NA	pretreatment by Sunds reactor; poor pretreatment performance; provided good experience with seed and main fermentation train; contaminant found, but too short a run to be a problem;
P950310CF	Batch	1400/ SSF	NA	NA	NA	NA	NA	pretreatment by Sunds reactor; poor pretreatment performance; problems with Sunds and feed addition systems made batch operation necessary; glucose used to sustain operation; contamination became a problem with longer term operation, contaminant type and source not identified
P950425CF	Cont.	1400/ SSF	25	26	NA	NA	49	pretreatment by APR throughout rest of CRADA work; poor pretreatment performance; continuous inoculum that was contamination free; many equipment problems; significant contamination problems in 9000-L fermenters (combined organic acids at 22 g/L); *lactobacillus* and *bacillus* species identified, no source identified
P951101CF Task 2	Cont.	1400/ SSF	25	17	52	NA	17	extremely poor pretreatment performance responsible for low yields; continuous inoculum; continuous feed additions (e.g., CSL); significant contamination problems that reduced ethanol yields, no long term effective control measure identified and no source identified, good test of equipment modifications, much improved PDU operability
P960122CF Task 3	Batch 9000 L	LNHST2/ SSCF	20	47	72	60	57	first use of blended feed; higher pretreatment severity and better sugar production; high concentrations of unconverted oligomeric glucose even with glucoamylase addition; contaminants detected, but not a problem
P960314CF Task 4	Cont.	LNHST2/ SSCF	25	36	62	20	37	lower pretreatment severity than Task 3 produced lower concentrations of monomeric xylose and less digestible cellulose and thus lower sugar conversions; continuous inoculation not needed; contamination controlled with Lactrol, no source identified; 6 dry tons of solid product collected

Run	Mode	Organism/ Process	Effective Solids Conc.[1] %	Ethanol Conc. (g/L)	Glucose Conv.[2] (%)	Xylose Conv.[3] (%)	Ethanol Process Yield[4] (%)	Comments/Lessons Learned
P960506CF Task 5	Cont.	LNHST2/ SSCF	2 5	37	73	26	47	more severe pretreatment than Task 4; better sugar yields; ; contamination always present, but quickly controlled with Lactrol; pretreatment feed and CSL identified as contaminant sources; saw conversion of oligomeric xylose to monomeric form; additional 6 dry tons of solid product collected.
P960506CF Task 5	Cont.	LNHST2/ SSCF	1 5	30	77	70	55	

NA -Not Available or Not Applicable

'Initial solids concentration based only on solids in the pretreated feedstock, actual measured level is lower due to conversion of sugars to ethanol

*Glucose conversion based on starch, cellulose, and galactan

[3]Xylose conversion based on initial monomeric and oligomeric xylose

[4]Yield based on ethanol produced divided by potential ethanol from starch, cellulose, and galactan for SSF with xylan added for SSCF

4.0 Technoeconomic Evaluation

The final Phase 3 milestone was to prepare a commercial design and cost estimate for the process developed under the CRADA that reflects the results from the Phase 3 program. Much of this work was carried out using Stone and Webster Engineering Company engineering standards, process design guidelines and equipment and costing information. The result is a robust commercial plant design which reflects the expertise and experience of the engineering and construction industry.

The following sections include the process design basis, a detailed process description, a description of the spreadsheet model constructed to evaluate the process, results of the evaluation of the platform case and results of sensitivity studies on the platform case.

4.1 Process Design Basis and Criteria

4.1.1 Design Basis

This section describes the design basis for the Reference Process Design Package prepared to evaluate the commercial application of the SWAN Biomass-to-Ethanol Technology to a blend of corn fiber and corn screenings as a feedstock. The corn fiber and cow screenings are assumed to be obtained from corn wet mills and the design site will be an integrated unit installed in a corn wet mill. This design reflects conditions and costs for locating the unit in a corn wet mill in Pekin, Illinois.

4.1.1.1 Scope

This design includes the systems listed below. Design documents, equipment lists, process flow diagrams, etc. uses the area designations for the units.

- System to transfer corn fiber and corn screenings from their origin in a corn wet mill to and including the SWAN pretreatment feed system (Area 1000).
- Feedstock pretreatment and fermentation preparation system (Area 2000).
- Fermentation system, also including the beer well and Nutrient Preparation Units (Area 3000).
- Ethanol recovery, dehydration and storage systems including distillation, dehydration with a molecular sieve unit and one day tank (Area 4000).
- Animal feed co-product handling systems comprising the separation centrifuges (Area 5000).
- Chemical Storage Systems to support this SWAN unit's operation (Area 8000).
- Chilled water system with a cooling tower (Area 9000).

All other utility and support systems for this design's operation are assumed to be from the existing plant's operations. They are accounted for through operating costs for utilities and other assessments.

4.1.1.2 Battery Limits

The battery limits of this design are the interface boundaries with the corn processing facility to which this plant will be added.

The process feedstocks, corn fiber and corn screenings will be taken from their present exit points in the wet mill and transported to this facility. The conveying units will be the equipment at the SWAN technology interface.

The product streams will interface with the larger plant at the battery limits as follows:

- Ethanol - the ethanol storage tank and rail/truck loading facilities will not be within the battery limits for this design.
- Animal Feed Coproduct - dry feed conveyors, dryers, storage bins and truck loading facilities for the final form of this product are not within the battery limits for this design.
- Carbon Dioxide - is a potential product but this design does not include a recovery system for it. It exits the battery limits to an atmospheric vent after scrubbing.

The utility interfaces are at the supply points to this design's utility distribution systems at the conditions given below.

The ethanol plant battery limits will be assumed to be on flat, unobstructed ground with physical dimensions determined by the most efficient preliminary layout.

4.1.1.3 Product Quality and Flows

Fuel Ethanol

This plant will produce fuel grade denatured ethanol meeting the following standard of composition:

Ethanol	95% (min)
Nonvolatile matter	5 mg/100 ml
Chloride ion content	40 ppm
Water	1.25%
Copper	0.1 ppm
Methanol	< 0.5%
Acidity	0.007%

The annual production will be 21.4 million gallons/per year of denatured ethanol. For design purposes, the hourly rate will be 2546 gallons/hour. The ethanol will be shipped in bulk trucks or rail tankers.

Animal Feed

The solids remaining after fermentation will be recovered and sold for their value as animal feed. Their composition cannot be precisely specified except for the moisture content which is a design criteria. The solids will generally be defined to fall within acceptable ranges of compositions depending on the feedstock and on the specific operating history of the fermentation.

The animal feed coproduct will contain 10 - 12% wt water and composed as calculated by the SWAN spreadsheet simulation material balance. It is anticipated that 37,400 pounds/hour will be produced without steepwater and 58,900 pounds/hour with steepwater.

The animal feed will be stored in bins after being dried as depicted in the process flow diagrams. Shipment will be in bulk solid container trucks, rail hopper cars normally used for animal feed. These animal feed solids may be pelletized before shipment if required.

The decision to pelletize will be made at a later date and specifications for the pellets will be issued at that time.

4.1.1.4 Onstream Days

This design case will operate for 350 days per year, and 24 hours per day. It will assume the production will be interrupted 10 days for a general annual maintenance and 5 days/per year for unscheduled outages. The unscheduled outages are specific to systems or equipment and will not result in a general shutdown.

4.1.1.5 Raw Materials

Feedstock

The feedstock for this design will be taken as it comes from the corn fiber and corn screening streams from the corn wet mill. This design case will be based on the average compositions of the materials used for the test runs at the National Renewable Energy Laboratory's (NREL's) Process Demonstration Unit (PDU) which are as follows:

COMPONENT	WEIGHT FRACTION (% WT)
Cellulose	7.13
Hemi-Cellulose: Xylan	7.49
Arabinan	4.84
Acetate	1.85
Starch	12.43
Lignin	3.38
Protein	3.42
Insoluble Ash	0.32
Water	54.30
Soluble Ash	4.83

The design flow of this feedstock will be 1,641 tons/day (750 dry tons/day).

Sulfuric Acid

Sulfuric acid for use of the APR will be purchased as concentrated liquid. Commercial grade sulfuric acid will be used with the following composition:

COMPONENT	WEIGHT FRACTION (% WT)
Sulfuric Acid	93.2
Water	6.8

The design case will consume 3,405 lbs/hour of concentrated sulfuric acid. Concentrated sulfuric acid will be received in bulk trucks.

Cellulase Enzyme

A cellulase enzyme solution will be used in the simultaneous saccharification and co-fermentation (SSCF) process. The enzyme solution will be added to the first SSCF fermenter vessel at the rate of 532 lbs/hour. In the design, this solution may be further diluted for addition. The enzyme solution for this design case is specified as a select strain of the fungus *Trichoderma reesei* and is an amber-liquid, active ingredient 77 FPU/ml min cellulase.

The material will be received as a liquid in 55 gallon drums or tank trucks.

Corn Steep Liquor

Corn Steep Liquor (CSL) will be used as a nutrient source for the yeast in SSCF. It is available commercially and will be characterized for this design by the CSL purchased and used in PDU test runs. The CSL is expected to come from the corn wet mill in a site-specific design. The design case CSL composition is:

COMPONENT	WEIGHT FRACTION (%WT)
Protein	15.3
Amino Acid, Mineral	7.5
Ash	4.8
Other	17.4
Water	55.0

Design case consumption of this CSL as purchased will be 1,487 lbs/hr.

Lime

This design case will use lime for neutralization of the hydrolyzate. It will be purchased in a convenient bulk solid form suitable for the lime mixing system for conversion to slake lime specified when the design is executed. It is likely that the lime will be purchased as the commercial grade specified as 95% CaO. In that form the design case will use 2,330 lbs/hr. It will ber received in tank trucks.

Glucoamylase Enzyme

This enzyme solution is used in the SSCF. It will be purchased in a commercial form identified as 250 Iu/ml G-Zyme 990. It will be used at a rate of 93 lbs./hr as purchased in the design case. It will be received in 55 gallon drums.

Caustic

50% caustic is used in the design to control pH in the fermenters and as the CIP cleaning agent. It will be received in tank trucks.

4.1.1.6 Inventories of Products and Materials

Products

Product inventories will be 7 days, a one-week operating cycle. Less than 7 days would be desirable if it is reasonable to expect that products can be shipped with such frequency.

Consumed Materials

Materials used by the plant should have a 7 day inventory for design unless that is unreasonably large for the normal resupply cycle. The determining factor for supply inventories will be economic optimum of the lowest unit cost for bulk shipments relative to the cost to store that size shipment until the next reliable delivery.

4.1.1.7 Utilities

- Natural gas is available at the following typical supply conditions:

  Temperature: 80°F
  Pressure: 100 psig

  Its composition is:

COMPONENT	MOLE FRACTION %
Methane	93.64
Ethane	3.49
Propane	0.07
Other Hydrocarbons	0.15
Carbon Dioxide	1.30
Nitrogen	1.35

  Gross Heating Values are:

  Dry 1014 Btu/CF
  Saturated 998 Btu/CF

- Process water will meet potable water criteria for this design and will be presumed to come from the existing plant's supply or from a municipal supply.
- Cooling water will be supplied from the existing wet mill and returned at the following conditions:

	SUPPLY	RETURN
Pressure at 20 ft. above grade	60 psig	25 psig
Temperature	86°F Max	100°F Max

- Steam will be available at 600 psig superheated by 25°F.
- Electricity is assumed to be available at the following conditions:

Voltage	Phases	Hertz	Use
4180	3	60	main supply
480	3	60	motor supply

- Chilled water will be provided by this design. Chilled water will be supplied at 50° F.
- Plant and instrument air will be available from the host wet mill at 100 psig, at ambient temperatures. Instrument air will be dried to dew point of -20 "F.
- Nitrogen will be available from the host wet mill at 75 psig and ambient temperature.
- Utilities will have the following values:

Utility	Units	cost ($/Unit)
Natural Gas	1000 mm Btu	2.85
Electricity	kWH	0.045
Potable Water	1000 gal	0.75
Sewage Costs	1000 gal	
Steam	mlbs	3.00

4.1.1.8 Site Data

Location:	Pekin, Illinois (Peoria)
Elevation:	652 ft. above (Sea Level)
Typical Ambient Temperatures	89 °F Max.
(Dry Bulb)	91 °F Min
Topography	Flat
Outdoor Design Temperatures	90 °F Max
	- 8 °F Min
Cooling Tower Design	
Max wet bulb operating temperature:	78 °F Maximum
	75 °F Minimum

4.1.1.9 Electrical Area Classification

The various plant areas have electrical classifications as follows:

Area No./Title	Electrical Classification
1000 Feedstock Handling	None
2000 Pretreatment	None
3000 Fermentation	None
4000 Distillation	Class I, Group D, Div 1 or 2
5000 Coproduct Handling	None
6000 Evaporation	None
8000 Chemical Storage	Class I, Group D, Div 2
9000 Utilities	None

4.1.1.10 Cleaning-in-place (Cip)

This plant will be designed in accordance with cGMP (current Good Marketing Practice) **codes.**

Contaminant microorganisms must not be allowed in the fermenters and the animal feed byproduct. All streams entering the fermenters, except the yeast seed, are sterilized by a process step or by a specific sterilizing unit. CIP equipment or the ability to disassemble and clean equipment easily must be provided from the point where the fermenter feeds are sterile to and including the fermenters. Similarly, the animal feed byproduct **must** be stored in equipment which can be maintained in accordance with GMP after being dried.

4.1.2 Design Criteria

System and equipment specific design criteria are part of the plant design basis. The overall intent of these criteria is to insure that plant will operate as planned for as long as expected. Criteria include overdesign factors, mechanical design criteria, sparing philosophy, standard calculation methods and many others. The most significant criteria for this process design are described below. Other standard criteria are provided in Appendix A-3 .

4.1.2.1 Overdesign Factors

Each service, equipment or system, will be designed with a specific overdesign factor to mitigate uncertainties in physical property data, design calculations, and plant operation. The overall intent is to ensure that the hourly production rate is maintained. Proposed overdesign factors for various services are:

SERVICE	OVERDESIGN FACTOR
Solids Handling	1.20
Pretreater (APR) System	Specified by vendor'
Fermenters (Vessels)	1.00
Heat Exchange Equipment Equipment types include: process/process reboilers, condensers, coolers, heaters, and cooling coils and plate coils inside other equipment	1.10 in addition to fouling factor
Distillation Columns	1.10
Evaporators	1.10
Filters/Centrifuges	1.30 of worst case
Dryers	Specified by vendor'
Compressors	1.05

SERVICE	OVERDESIGN FACTOR
Pumps	
Transfer	1.10
Reflux	1.20
Vacuum Pumps	1.10

'The factor for these systems will be set by the vendors as a result of design studies and integrated operating strategies to be determined by those studies. The most lkely vendor candidates will be consulted for this design case.

4.1.2.2 Mechanical Design Criteria

Internal Finishes

The internal finishes for equipment surfaces in contact with process streams must meet industry standards for the materials in those streams. It is extremely important that all surfaces that contact materials that go into the SSCF fermenters meet standards for industrial fermentation. This includes finishes and specification of the equipment to be used. Regular and, at times, very frequent sterilization of equipment handling such materials will be required. Their design must reflect this requirement.

The following table indicates which areas must meet fermentation standards and, where possible, what finish specifications apply.

AREA NUMBER	AREA TITLE	FERMENTATIONS AND FINISH SPECIFICATION (YES/NO)
1000	Feedstock Handling	No
2000	Pretreatment	Yes (After APR)
3000	Fermentation	Yes
4000	Distillation	No
5000	Coproduct Handling	No
6000	Evaporation	No
8000	Chemical Storage	No
9000	Utilities	No

Equipment Spares

All essential transfer equipment will be spared, have features, or have strategy that will allow resumption of operation soon enough not to interrupt downstream operations. All filters and pumps that are in continuous service will be spared. Where feasible, cost effective and/or required for emissions and safety; spares will be installed. If the spare is needed, but not installed, the design will provide for rapid and safe installation of the spare from storage.

The Pretreatment Section will have 2 APR machines installed each with 75% of total throughput capacity. APR Support Systems will be spared as required based upon the cost-effectiveness and process and mechanical design requirements.

Turndown and Flexibility Factors

This design will have an overall turndown factor of 75% which reflects the operation of 1 APR(s) out of the 2 installed. Vessels with residence time requirements must have internals and other features in place that will accommodate the reduced operating volumes for turned-down operations.

Materials of Construction

The materials of construction will be those present in the NREL PDU, where the test runs were made, except when other experience indicates satisfactory alternatives. The results of a material study may be available for use in the design and will be distributed when available. A notable exception to matching PDU materials will be for the fermenters. The design fermenters will be large **and** will be field-erected. Whereas the PDU uses stainless steel, the design should use coated or lined carbon steel for the fermenters.

The following table indicates the materials of construction presently demonstrated and specified by plant area.

AREA NUMBER	AREA TITLE	MATERIALS	COMMENT
1000	Feedstock Handling	CS	As normally supplied by vendor for the corn fiber
2000	Pretreatment ▪ General APR	316SS See comment	Per CRADA Phase 3 Report
3000	Fermentaton ▪ General Fermenters	304 ss Lined Carbon Steel	Erected and lined in field
4000	Distillation	304/316 CS	
5000	Coproduct Handling	SS/CS	
6000	Evaporation	304 cs	
8000	Chemical Storage Sulfuric Acid Lime Solution Cellulase Solution Glucoamylase Solution Caustic	 cs CS SS SS CS/SS	
9000	Utilities	Per normal vendor supply	

4.1.2.4 Other Plant Design Factors

Detailed information on pressure calculations, vessel sizing, hat exchanger design, and pump specifications are provided in Appendix A-3, Section 4. Civil, structural, electrical, and piping design information is provided in Appendix A-3, Section 6, and Process Control Philosophy in Appendix A-3, Section 1.

4.2 Process Description

The SWAN Biomass to Ethanol process described here assumes the integration of the SWAN Biomass process with an existing corn wet mill (approximate capacity of 150,000 bushels of corn per day) which would supply the corn fiber and screenings used in this description. Additional fiber and screenings could be supplied by other corn wet mills in the immediate geographic area to make the biomass process capacity of such size as to better enhance its economics.

The SWAN Biomass to Ethanol technology uses a proprietary pretreatment step that hydrolyzes the feed while minimizing by-product formation, a proprietary yeast that is capable of fermenting both hexose and pentose sugars, a simultaneous saccharification and fermentation and a low cost enzyme. These four process innovations distinguish the SWAN ethanol process from other biomass to ethanol processes.

The following sections provide descriptions of the main process steps of the biomass to ethanol process. Because this plant has been integrated with an existing plant the inside the battery limits (ISBL) steps are limited to the following process steps:

Feed Handling	1000 area
Pretreatment	2000 area
Fermentation	3000 area
Distillation	4000 area
Co-Product Handling	5000 area
Chemical Storage	8000 area
Chilled Water System	9000 area
Cooling Tower Water System	9000 area

This description assumes an adequate supply of the following out-side the battery limits (OSBL) utilities (9000 areas) and services:

High and Low Pressure Steam (600, 150 and 50 psig)
Plant and Instrument Air (100 psig)
City or Process Water
Electricity
Waste Water Treatment (both anaerobic and aerobic - 7000 area)

4.2.1 Feed Handling (1000 Area)

Wet corn fiber (approximately 35 to 40% solids) from the existing corn wet mill fiber presses (or supplementary wet fiber from other mills) is transferred to the biomass to ethanol plant by screw conveyors. It is stored briefly in the fiber storage bin which serves as a two hour surge between the existing wet milk and

the ethanol plant's pretreatment step. The wet fiber from the storage bin is first passed by a magnetic separator to catch any trash ferrous metal before it is transferred to the pretreatment step by a weigh belt and transfer screw conveyors. The fiber storage bin is equipped with a live bottom and discharge auger conveyor to prevent bridging inside the bin. The weigh belt measures the flow rate to the pretreaters and controls the output of the plant.

Screenings from the wet mill's corn receiving area are transferred directly (no intermediate storage) to the pretreaters by a separate weigh belt and transfer conveyors system. Screenings are a supplemental feed to the process and are fed as they become available. However the fiber to screenings ratio of most wet mills will be approximately 8.5 to 1.0. A higher ratio could be used but the pretreatment and fermentation process conditions have been established on this ratio. A magnetic separator is used to remove any trash ferrous metals, which could damage the pretreaters, from the screenings before they are fed into the pretreaters.

The Feed Receiving and Storage process is shown in the following PFD:

Corn Fiber/Screenings Feed • PFD1101

4.2.2 Pretreatment (2000 Area)

The first of the SWAN Biomass to Ethanol process proprietary steps is pretreatment. Fiber and corn screenings are fed directly to the pretreater. In the pretreater, the five-carbon sugar polymers contained in the biomass are hydrolyzed along with the starch and a small amount of the cellulose. The hydrolyzate is then neutralized using slake lime, the solids content adjusted with thin stillage (back set) from the co-product handling area and then cooled before continuously being fed to the first fermenter. The pretreater operating conditions are critical in determining sugar conversions and minimizing the production of unwanted by-products, such as 5-hydroxymethyl furfural (HMF) and furfural, which represent yield loses as well as function as inhibitors to the fermentation process.

The Pretreatment step is shown in the following PFDs:

PFD 2 101 Pretreater
PFD 2102 Neutralization

Pretreatment

In the pretreatment step, fiber and screenings are fed directly to one of two pretreaters. With the feed, sufficient dilute sulfuric acid is also fed into the pretreater. Concentrated sulfuric acid (93 %) is diluted with thin and is stored in the dilute acid storage tank. Dilute acid is fed to the fermenters (for pH control) with a centrifugal pump and to the pretreaters using a high pressure diaphragm pump. High pressure steam (400 to 450 psig) is fed to the pretreater and used to control the pretreatment pressure, which raises the pretreater temperature (depending on feed composition).
The steam combined with the dilute acid hydrolyzes the feed. The pretreater (a proprietary mechanical hydrolyzer) converts the starch and cellulose into intermediates that through additional enzymatic reactions can be converted to fermentable forms of glucose. The hernicellulose fraction of the biomass (mostly xylans) is converted by the pretreater to xylose. The glucose and xylose fractions are also converted to unwanted by-products, HMF and furfural. These by-products are sources of yield losses and are inhibitors to the fermentation process. The hydrolyzate containing these converted fractions is next fed to the flash cooling step.

Flash Cooling

The flash cooling step is a single stage flash which cools the hydrolyzate, allows the recovery of low pressure steam and the collection of most of the by-products (HMF and furfural) produced by the hydrolysis step. These by-products will function as inhibitors to the fermentation step if not removed. The hydrolyzate leaving the pretreater discharges into the low pressure (LP) flash tank which operates at a pressure of approximately 50 psig. The LP flash vapor is used to preheat the dilute sulfuric acid (using a graphite heat exchanger) and is condensed and sent to the waste water treatment system. The cooled hydrolyzate (now at about 60 C) flows by pressure to the neutralization step.

Neutralization

In the neutralization step the hydrolyzate is neutralized, diluted and cooled for feed to the first fermenter. Lime is delivered by truck and unloaded into the lime storage tank. It is then mixed with water in the slake lime mix tank to form a slake lime mixture of calcium hydroxide. The slake lime is added directly and continuously to the suction side of a mixing pump to neutralize the hydroylzate from a pH of 0.8 - 1 .O to a pH of approximately 5.0. Thin stillage as back set is also continuously added to the suction side of the mixing pump to dilute the hydrolyzate from approximately 30 to 32 % solids (from the pretreaters) to 25 % solids for feed to the first fermenter. Thin stillage is used for this purpose to recycle any dissolved protein and to minimize water usage and waste water treatment costs. The dilute and neutraiized hydrolyzate is then cooled to approximately 30°C with a series of plate and frame heat exchanger and is continuously pumped to the first fermenter.

During start-up and periods of upsets at the pretreaters the hydrolyzate can be fed to the waste tank where it is collected, cooled and diluted. This is done to prevent sending partially converted or non-sterile hydrolyzate to the fermentation system causing up sets or possible bacterial contamination. The waste hydrolyzate can then be pumped to the waster water treatment plant for neutralization and treatment.

4.2.3 Fermentation (3000 Area)

The SWAN Biomass fermentation process utilizes a simultaneous saccharification and co-fermentation (SSCF) process. In the SSCF fermenters, enzymes (glucoamylase for the starch and cellulase for the cellulose) and a proprietary yeast are added to the hydrolyzate to promote the simultaneous reactions of saccharification and fermentation. Saccharification of the dextrin (from the starch component of the biomass) and cellulose takes place along with the fermentation of the glucose and xylose (contained in the hydrolyzate) to form ethanol. The by-product, carbon dioxide, exits the fermenters and is collected, scrubbed with water to remove any entrained ethanol and then vented to the atmosphere or transferred to a recovery unit off-site. Beer broth from the fermenters is transferred to the beer well where it is then filtered to separate an ethanol rich liquid stream that is recycled back to the first fermenter. The rest of the broth for the beer we13 is fed to the distillation system. The nutrient for the yeast, corn steep liquor (CSL), is first sterilized before being used in the first fermenter.

The fermentation process is shown in the following PFDs:

SSCF Fermenters -	PFD3201
Beer Well -	PFD3202
CSL Sterilization -	PFD3401

SSCF Fermenters

Hydrolyzate is continuously fed to the first fermenter of a three stage continuous fermentation train. Co-reactions of saccharification and fermentation take place in the first fermenter. Enzymatic reaction (saccharification) of the dextrin and cellulose to glucose occur simultaneously with the fermentation of the glucose and xylose sugars. The reaction products are ethanol, carbon dioxide and other by-products of lesser significance. The fermenters have been sized (1 ,000,000 gallons) to give an individual fermenter residence time of 24 hours and a total residence time of 72 hours.

The first fermenter is equipped with adequate agitation to maintain a suspension of 25 % solids, an external shell and tube heat exchanger cooled with chilled water to maintain a temperature of 30 C, pH adjustment capability using dilute caustic and sulfuric acid to control the pH to 5, sterile water and air supplies, circulation pump, carbon dioxide vent and feed lines for cellulase, glucoamylase, CSL, recycle ethanol, yeast/glucose (from trucks) and hydrolyzate. On a continuous basis neutralized and cooled hydrolyzate is fed to the first fermenter. To convert the cellulose to glucose and any unreacted xylans to xylose, cellulase is continuously added at a rate of cellulose at a concentration of 77,000 IFPU/liter. Glucoamylase is continuously added to the first fermenter to convert the dextrin (converted from the starch in the pretreater) to glucose. CSL is fed continuously to add nutrients for the continuous growth of the yeast cells. The CSL is added at a rate of 0.43% of the fermenter feed at a concentration of 9% CSL. Small amounts of dilute caustic and sulfuric acid are added to control the pH of the fermenter at 5. A side stream from the heat exchange circulation loop is continuously sent to the second fermenter. Yeast is normally prepared and fed batch wise at the start of the fermentation. It can be added during the fermentation step if the fermenter cell mass drops below acceptable limits. The second and third fermenters are all equipped in the same way with pH control, external shell and tube heat exchangers, circulation pump and carbon dioxide vent. A portion of the ethanol rich liquid stream from the cross flow filter is recycled to the first fermenter to increase the ethanol content of the final fermentation broth to 7 to 8 % (w/w).

Beer Well

Flow from the third fermenter is collected in the beer well (same size and configuration as the fermenters) where it is then continuously fed to a cross flow filter. The cross flow filter is a membrane filter which separates the fermentation broth into an ethanol rich liquid stream (containing approximately 22 % of the total broth with about 8 to 9 % ethanol) and a "solids" rich stream (containing approximately 78 % of the total broth). The ethanol rich liquid stream is recycled to the first fermenter. The "solid" rich stream is mixed with any of the ethanol rich liquid stream that is not recycled and fed, as beer. to the beer column in the distillation system. Normally 100 % of the ethanol rich liquid stream is recycled.

All the carbon dioxide that is generated in the three fermenters is sent to the carbon dioxide scrubber. The carbon dioxide scrubber uses water to wash out any ethanol that might be entrained in the carbon dioxide and sends this wash stream back to the beer well. The clean carbon dioxide is either vented to the atmosphere or sent to a carbon dioxide collection facility off-site.

CSL Sterilization

Corn steep liquor (CSL) from the existing wet mill is transferred to the CSL sterilization area where it is continuously sterilized by heating and holding at temperature for a specific length of time. CSL is taken from the wet mills steep water holding tanks and continuously pumped to the CSL hold tank. The hold tank functions as a surge tank between the wet mill and the ethanol plant and can also function as a dilution tank. The preferred CSL concentration is 9 % but either light or heavy steep water can be used (heavy steep water would have to be diluted down from approximately 50 % to the 9%. light steep water which is between 7 and 11 % could be used as is). The CSL is continuously fed to the sterilization hydroheater after being preheated in the preheater and flash condenser to a temperature of approximately 80 C. The hydroheater heats the CSL

to about 135 to 140 C using the direct introduction of low pressure steam (35 psig). The CSL at 135 C then enters the sterilization coil which gives a retention time of approximately 1 minute. These conditions, 135 C for 1 minute, is enough to sterilize the CSL. The CSL is then flash cooled to approximately 90 C in the CSL flash tank. A small vacuum is required in the flash tank to flash off enough water to maintain a 9 % CSL concentration. The sterilized CSL is then held in the sterile CSL tank for continuous feeding to the first fermenter. During transfer to the first fermenter the sterile CSL is cooled in the preheater and sterile CSL cooler to a temperature of about 35 C. The vapors from the flash tank are condensed in the CSL flash condenser and the condensate is sent to the waste water treatment system, Any noncondensables pass through the vacuum pump and are vented to the atmosphere.

4.2.4 Distillation (4000 Area)

The distillation and dehydration area consists of two main columns and two molecular sieve beds. After preheating, the beer goes to the top of the beer column where it is stripped of ethanol and degassed of carbon dioxide. The beer column bottoms (whole stillage) are collected, cooled and sent to the co-product handling area (5000). The ethanol vapor from the top of the beer column flows to the rectifying column where it is concentrated to near the azeoptropic point. A portion of the overheads vapor from the rectifying column is condensed and used as reflux. The remaining vapor from the rectifying column is sent to the molecular sieve for dehydration. The bottoms of the rectifying column are sent back to the beer column to remove the last traces of ethanol.

The final dehydration of the ethanol to produce anhydrous fuel grade alcohol is achieved in the molecular sieves by selective adsorption in the vapor phase. Water is adsorbed on the sieves while anhydrous ethanol passes through the bed. The adsorbed water is removed during a regeneration step and sent back to the distillation system.

The preferred method of regeneration of the molecular sieve beds is a "pressure swing" system which requires virtually no heating in the process other than to superheat the feed and purge vapor to offset heat losses, The pressure swing will be achieved with a vacuum system. Adsorption takes place under positive pressure while regeneration takes place under vacuum.

The quality of the ethanol from the distillation step is constantly monitored by collection of the dry ethanol in one of two day tanks. When the product meets specification, it is transferred to the denatured ethanol storage tank, which has the capacity to store approximately 7 days of production. Five percent gasoline is metered into the line to the denatured ethanol storage tank and functions as the denaturant. From the storage tank, the ethanol is loaded into either trucks or rail cars for shipment. Emissions are minimized by using a floating roof storage tank and vapor recovery units on other tanks and loading arms.

The distillation process is shown in the following PFDs:

Beer Column -	PFD4101
Rectifying Column -	PFD420 1
Molecular Sieves -	PFD4601
Alcohol Storage & Loading -	PFD4801

Beer Column

Beer is preheated and continuously feed to the top of the beer column. The beer column strips the ethanol from the beer leaving whole stillage. Beer from the cross flow filter is pumped through a series of heat

exchangers to preheat the beer before it enters the beer column. The first exchanger is the beer/stillage exchanger where the beer is preheated to a feed temperature of about 95 C. The second preheater is used to supplement the beer/stillage exchanger and is normally used during start-up. The beer column contains specially design trays to allow the downward flow of the solids containing stillage and also functions as a degasser for removing carbon dioxide for the beer. The ethanol vapors leaving the beer column (at about 105 C) flow to the bottom of the rectifying column. The whole stillage (at about 11.5 C) from the beer column, containing approximately 15 to 20 % total solids and only a trace of ethanol, is cooled in the beer/stillage exchange to about 48 C and is collected in the whole stillage tank for further processing. The beer column reboiler is the only distillation area that normally uses steam.

Rectifying Column

The overheads from the beer column are dehydrated almost to the azeotropic point in the rectifying column. No heat input is necessary in the rectifying column as the beer column overheads contain enough heat to dehydrate the ethanol. Rectifying column overheads (at about 87 C and a ethanol concentration of 94 %) are split with a small side stream going to the RC reflux condenser and functions as reflux for the rectifying column. The remaining portion of the overheads flows to the molecular sieve beds for further dehydration. A side stream of fuse1 oil (mostly amyl, isoamyl and propyl alcohols) is taken from near the top of the rectifying column, condensed, and then remixed with the anhydrous ethanol in the ethanol storage area. The rectifying column bottoms (at about 105 C) are fed back to the top of the beer column to remove all the remaining ethanol.

Molecular Sieves

Ethanol vapor from the rectifying column is superheated to about 115 C with steam and fed into one of two molecular sieve beds. One bed operates while the other regenerates using a portion of the anhydrous vapor product. The dehydrated ethanol (about 99.5 % ethanol) is used in the regen preheater to preheat the dilute ethanol regenerate stream before it is sent to the rectifying column, then condensed, cooled and sent to an alcohol day tank. Regeneration results in a dilute ethanol stream which is condensed and collected in the surge drum and sent back to the rectifying column to recover the ethanol.

Alcohol Storage and Loading

The dehydrated ethanol flows to a new alcohol day tank where it is monitored by taking periodic samples to see if it meets the required specification for fuel grade ethanol. If the ethanol does not meet specifications it is returned to the rectifying column for reprocessing. If the ethanol meets specifications it is pumped to the existing large denatured alcohol storage tank. While being pumped to the storage tank , it is mixed with gasoline (5 %) to form denatured ethanol. The anhydrous ethanol can be loaded into tank trucks or tank cars for shipping. Hydrocarbon emissions are a concern in this area and precautions are taken to minimize these emissions. Vapor recovery unit is installed on the new alcohol day.

4.2.5 Co-Product Handling (5000 Area)

The whole stillage from the beer column contains considerable amounts of dissolved and suspended solids which are recovered in the co-product handling area. These solids contain valuable protein and can be used as animal feed. The suspended solids (co-product) are separated from the liquid portion, which contains the soluble solids and is called thin stillage, by decanting centrifuges and dried using rotary steam tube dryers. The dry co-product is stored and shipped as a high protein animal feed for poultry, swine and cattle. The thin stillage is fairly free of soluble proteins and is sent directly to the wastewater treatment step.

The co-product handling process is shown in the following PFDs:

Co-Product Separation -	PFD5101
Co-Product Drying -	PFD5201
Co-Product Storage -	PFD5401

Co-Product Separation

Whole stillage contains about 5 to 7 % soluble solids and about 10 to 13 % suspended solids. A considerable amount (about 30 %) of valuable protein is contained in the suspended solids. The suspended solid are separated from the liquid stream (thin stillage) by feeding the whole stillage to two new decanter centrifuges. These are solid bowl centrifuges that separate solids from liquids. These suspended solids are difficult to separate causing the "dry" cake from the centrifuge to contain only 25 to 35 % solids. The thin stillage contains some of the suspended solids but is not processed to recover these solids. The thin stillage is collected in the thin stillage storage tank. Part (approximately 20 to 50%) of the thin stillage is recycled back to the pretreatment step as diluent water for the sulfuric acid and to adjust the solids content of the hydrolyzate. This is done to reduce water usage and waste water treatment costs. The remaining thin stillage is sent to the waste water treatment plant. The "dry" cake from the centrifuges is transferred to the drying area by new screw conveyers. Before it reaches the drying area the cake is mixed with concentrated steep water (about 50% solids) from the existing wet mill.

Co-Product Drying

Much of the existing drying system is reused. The "dry" cake (along with the concentrated steep water) is screw conveyed to the wet cake mixer (existing). Here the cake is mixed with dry co-product recycle to lower the moisture level of the feed to the drier to around 25 %. To accomplish this a recycle to wet cake ratio of 3 or 4 (depending on the wetness of the cake) to 1 is necessary. The 25 % moisture cake is fed to two existing rotary steam tube dryers. The dry -product exits the dryers at 80 C and at a moisture content of approximately 10 to 12 %. The co-product is pneumatically conveyed, which also serves to cool the co-product to about 40 C, to the existing co-product storage bin.

Vapors from the dryers (at about 105 to 110 C) are passed through the wet cake dryer scrubber. This scrubber removes all the dust particles from the dryers and any other fumes or odor containing vapors. The scrubber uses fresh water and the scrubbing water is discharged to the waste water treatment plant.

Co-Product Storage

The cooled co-product is stored in the existing co-product storage bin and transferred by existing drag conveyors to one of two loading options. Co-product can be loaded into trucks or rail hopper cars for shipment to the animal feed lots or animal feed formulator. All the transfer drag conveyors are connected to an existing central dust collection system which recycles the dust back into the finished co-product. This will minimize any housekeeping problems or explosion hazards with the dust.

4.2.6 Chemical Storage and CIP

Storage for all the chemicals used in the process are provided this includes the following:

Cellulase
Glucoamylase
Sulfuric Acid
Caustic

In order to keep the process microbiologically clean and to remove residues from heat exchangers, vessels, and process piping an automatic cleaning-in-place (CIP) system is supplied.

The chemical storage and CIP systems are shown in the following PFDs:

Chemical Storage - PFD8101
Chemical Storage CIP - PFD8401

Cellulase (if not made on-site) is received in tank truck quantities and glucoamylase in drums and are pumped into their respected storage tanks. These tanks are agitated but not heated and have transfer pumps for charging to the first fermenter. 50% Caustic is received by tank trucks and unloaded into its agitated and heated tank. It is diluted in the process area for use if needed and directly to the CIP system. 93 % sulfuric acid is received in tank trucks and unloaded into its storage tank. This tank is agitated and equip with a desiccant vent dryer to prevent moisture from entering the tank. The concentrated acid is diluted to 8 % in the pretreatment area.

Cellulase enzyme if produced on-site will be supplied from an over-the-fence enzyme manufacturing facility that uses highly proprietary SWAN Technology.

Figure 4.5
Net ethanol Cost Versus Capacity

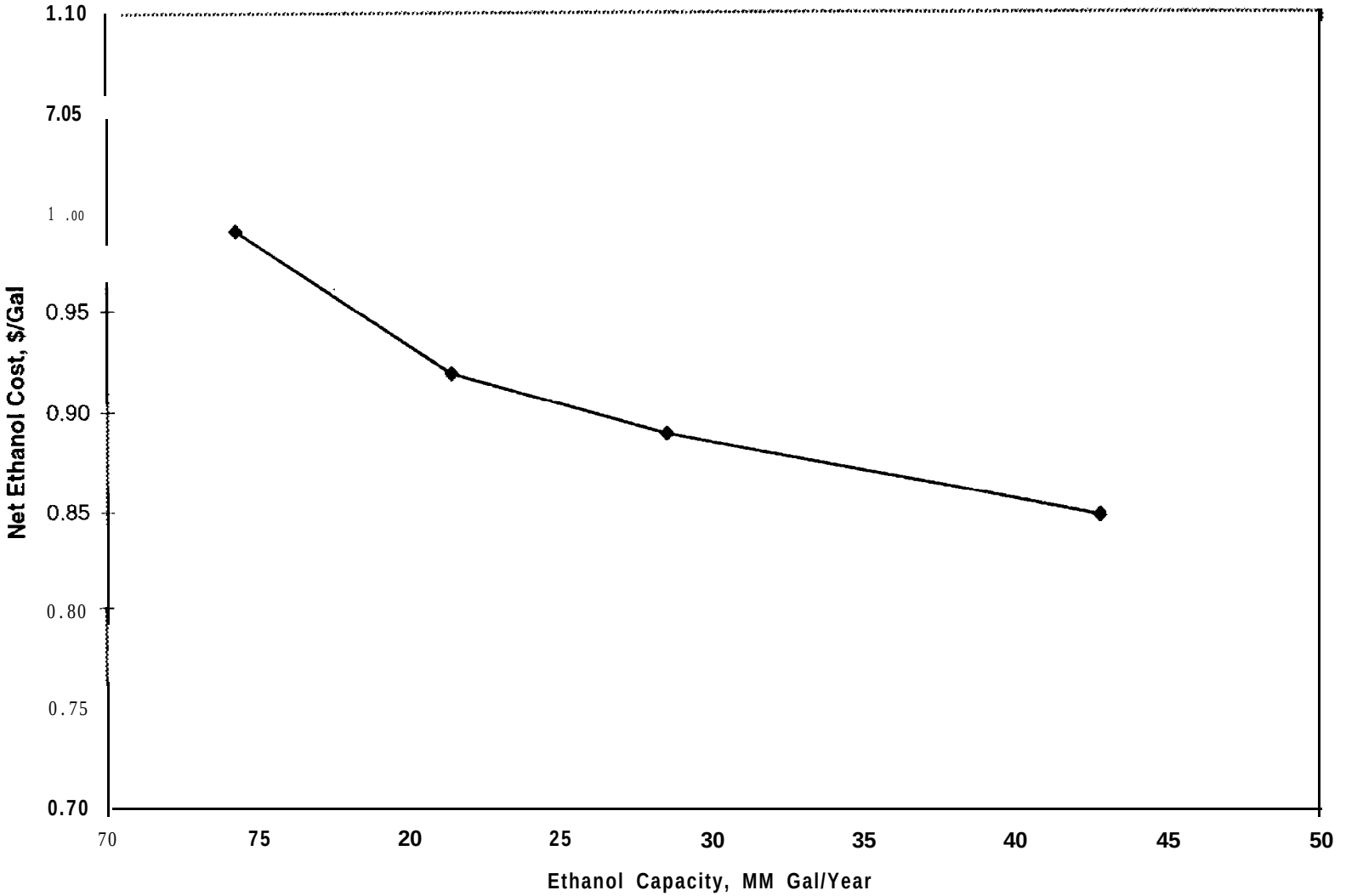


Figure 4.4
Fermentation Residence Time Versus Net Ethanol Cost

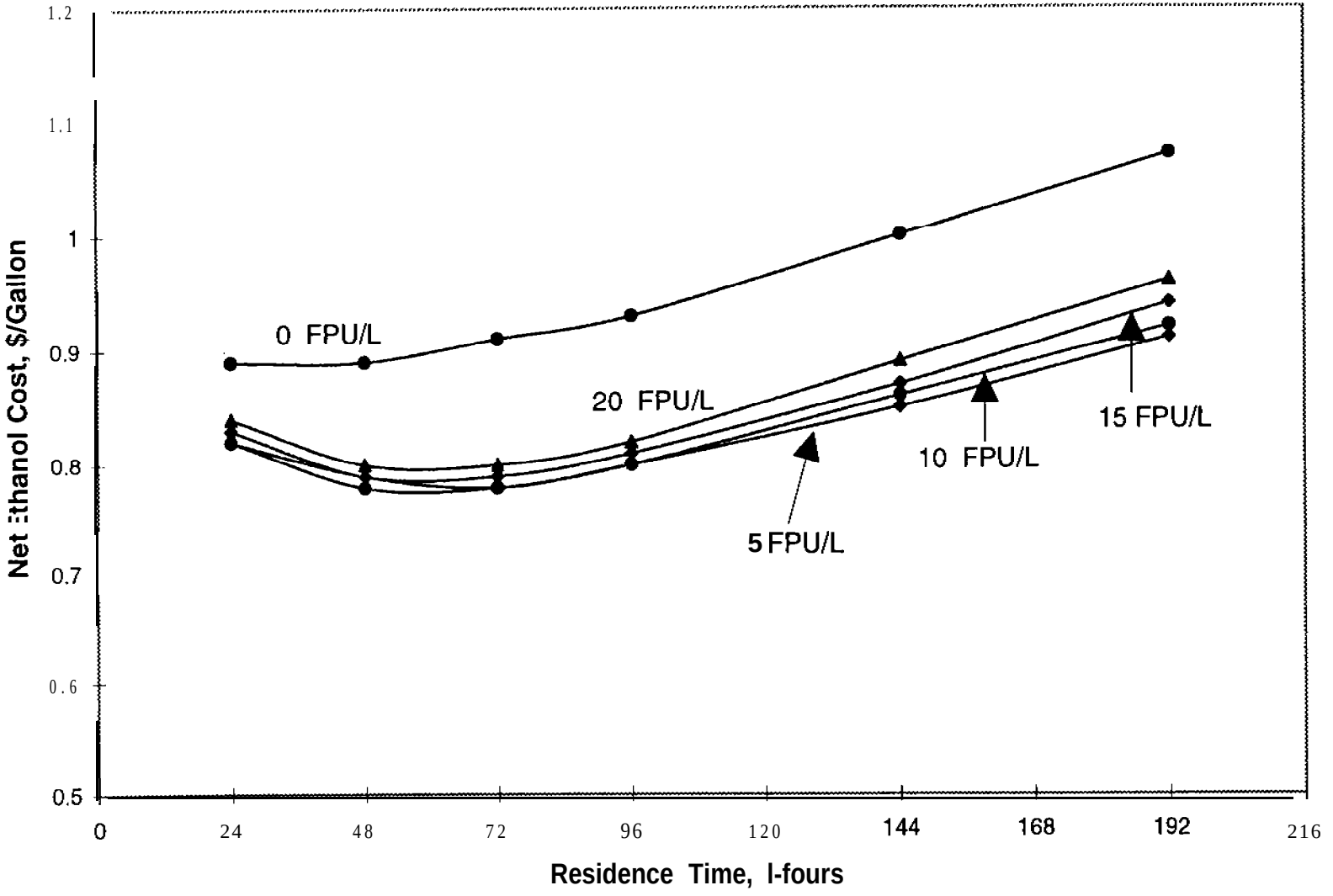
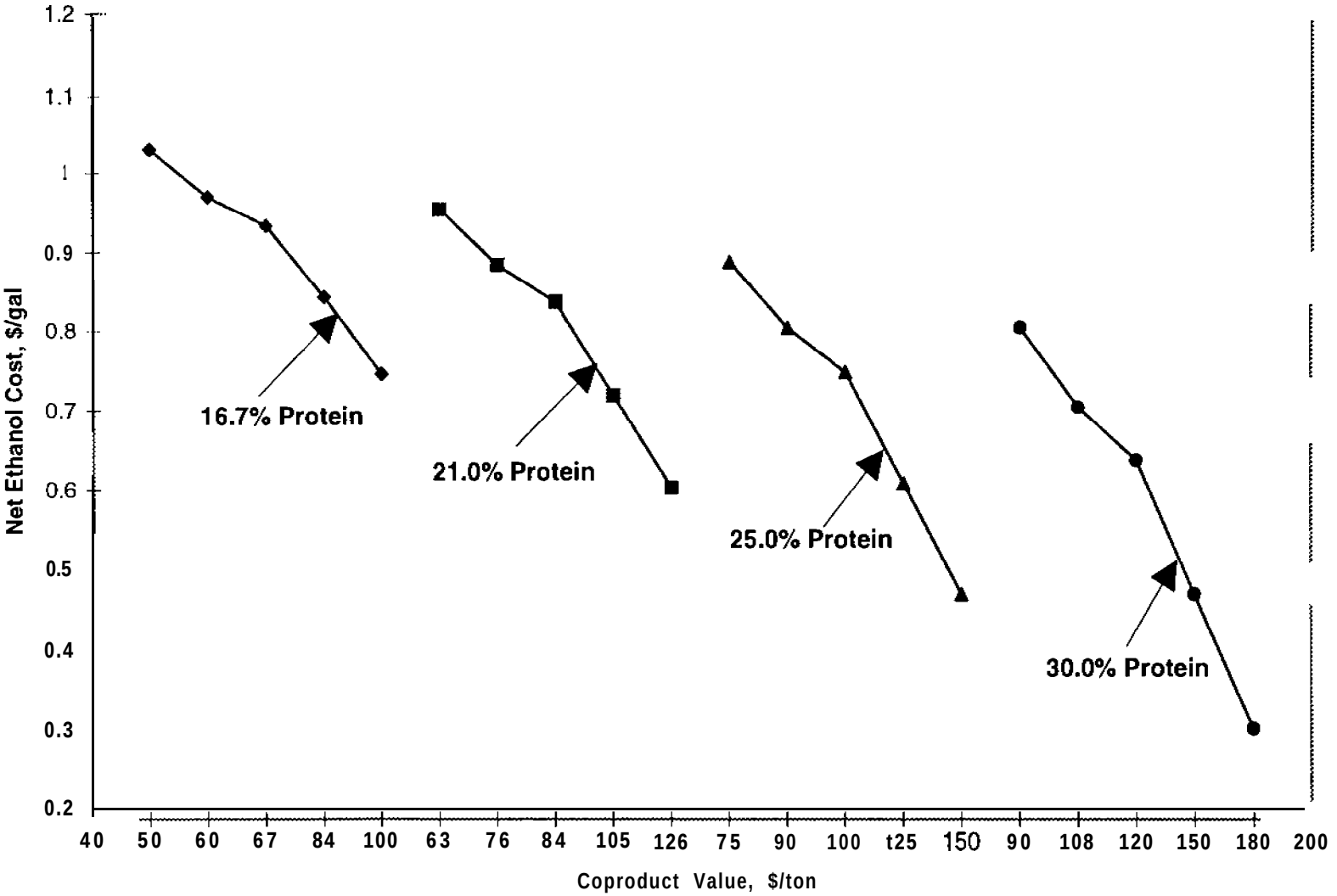


Figure 4.3
Coproduct Value Versus Net Ethanol Cost



The CIP system is a two tank fully automated system using caustic as the cleaning agent. The condensate rinse tank is used to collect condensate and fresh water to be used in both the pre-rinse and post-rinse steps. It is neither heated nor agitated. The caustic tank is used to dilute the 50 % caustic down to 1 to 5 % caustic and to heat it to between 55 to 80 C. A waste tank is also provided to collect all the waste streams before they are sent to the waste water treatment plant. This tank helps regulate pH swings in the waste water treatment system and prevent discarding wash solutions before they have been spent. The typical CIP cycle would start with a pre-rinse using the water in the condensate rinse tank. This rinse would be sent through all the CIP locations and then to the waste tank. The purpose of this rinse is to remove the loose material in the system. The second step is a hot alkaline wash using the dilute caustic from the caustic tank. This wash is circulated through the system and then back to the caustic tank. This circulation period will range between 30 to 60 minutes depending on the temperature used and the extent CIP is needed. During the alkaline wash the condensate rinse tank is filled with fresh condensate or water. The alkaline wash is then followed by a post-rinse wash using the water in the condensate rinse tank. This wash is circulated back to the condensate rinse tank for a few minutes and will be used for the next pre-rinse cycle. Between cycles the caustic tank is adjusted for both temperature and caustic concentration. The caustic tank is purged from time to time.

4.2.9 Utilities (9000 Area)

As mentioned in the Basis of Design , it has been assumed that all utilities will be available from the existing wet mill. However the SWAN Biomass to Ethanol process requires a lower than normal fermentation temperature (30 C) which makes it impossible to cool the fermentation broth using cooling tower water at some plant locations. To accomplish this a 10 C chill water system is necessary. This system would include a packaged chiller unit and a surge tank and transfer pump. This system would supply 10 C water to all the fermenters and the hydrolyzate cooler in the pretreatment area. The chiller system will use large quantities of cooling tower water and thus this design includes a separate cooling tower.

The cooling tower water and chilled water systems are shown in the following PFDs:

Cooling Tower Water System	PFD9401
Chilled Water System	PFD9501

4.3 Spreadsheet Model

An Excel spreadsheet model was originally developed by Amoco researchers to examine the design and cost of commercial facilities for converting the biomass in municipal solid waste (MSW) to ethanol. Both reaction kientics and a more detailed breakdown of capital costs were added to the spreadsheet during Phase 2 of the CRADA, as reported in the Phase 2 Final Report. The original spreadsheeet was the basis for the model developed during Phase 3; the added complication and increased calculational time added in Phase 2 was judged to be more of a hindrance than a help. The last major revision to the spreadsheet was made **April,** 1996.

The current version of the model is written to examine corn fiber as a feedstock for the production of ethanol. The model includes a summary sheet, a four-page input parameter section, the calculated stream flows, and the resulting capital and operating costs. The input parameters include positioning a number of switches to choose between alternatives to the design. After setting the input parameters, the model is run in an iterative fashion, varying enzyme dose, SSCF residence time, internal recycle rates and other parameters, until the lowest cost for ethanol is found.

The model requires that the yields and conversions be determined elsewhere, and these values serve as inputs for the model optimization. For Phase 3, the yields and conversions were determined in a separate computer model, and tables of these values were made available for the design and cost calculation.

43.1 Spreadsheet Process

The corn fiber and corn screenings to ethanol plant is assumed to be located at Pekin Energy's wet milling facility in Pekin, Illinois, and is to be built adjacent to the existing plant. The fiber/screening plant would share a number of utilities and other facilities already in operation at the Pekin plant. The block flow diagram for the model is shown in Figure 4.1. The flow stream numbers of the block flow diagram correspond to streams numbers in the spreadsheet model material balance which is included in the appendix at the end of this section. Corn fiber/screenings are taken from the existing fiber presses in the wet milling operation and fed into the pretreater APR with steam and acid. The exit stream from the pretreater is flashed cooled. A mixer is used to push the slurry into the SSCF feed pump and functions as the mixer for the lime and recycle streams. There are multi stages of SSCF in series, each with twenty four hours residence time. Corn steep liquor (CSL), acid, base, enzyme and yeast are added to the first SSCF fermenter. Carbon dioxide from the fermenters is scrubbed to recover evaporated ethanol. Beer from the last fermenter is sent over to a cross-flow filter, and some of the liquid which contains ethanol is recycled to dilute the pretreated slurry. The second purpose of this recycle is to raise the ethanol concentration in the distillation feed so that distillation costs remain reasonable. The remaining beer from the last fermenter is sent to distillation where the ethanol is removed and then dried, using molecular sieves. Fuse1 oils from the distillation system and denaturant are added to the product, which is then sent to storage. The water and solids from the distillation bottoms are routed to a solids separation step which uses a centrifuge to separate the water and solids. The solids are sent to dryers in the existing corn mill plant. Some of the centrate is recycled to dilute the pretreated solids, and the remainder is also sent to waste water treatment in the existing plant. The waste water goes through an anaerobic digestion, which produces methane used as a process fuel.

To have the spreadsheet model generate the platform case, the basic assumptions needed to calculate mass balances, determine equipment sizes and estimate capital and operating costs for the add-on plant are:

Feed

1. The feedstock is corn fiber, with corn screenings added. The ratio (typical of most wet corn mills) is 8.5 parts fiber to 1 part screenings on a dry basis. This was the feedstock used for most of Phase 3.
2. The feed rate is 750 dry tons/day.
3. The feedstock contains 45.7% solids and 54.3% water, as measured -in Phase 3.
4. The dry feedstock composition is:

27.20 wt % starch
15.60 wt % cellulase
16.40 wt % xylan
10.60 wt % arabinan
4.04 wt % acetate
7.40 wt % lignin
7.50 wt % protein
0.70 wt % insoluble ash
10.56 wt % soluble ash

These values are weighted averages over Task 5 in the PDU (and are given in Table 4.2 as "Modified NREL." This composition may underestimate protein concentration.

5. Feed is valued at $0.20/lb protein, based on a corn price of $2.50/bushel. A correlation was developed (see Section 3.4) relating historical values of corn fiber to corn price.

Pretreatment

6. Feed goes to pretreatment directly from the existing plant equipment, with no addition or removal of water. There is minimal interstage storage. Fiber and screenings are few separately, but simultaneously, into two APR units in parallel.

7, Sizing of the APR units is based on PDU results:

8

Steam supply is 600 psig saturated
steam. These conditions were established in the PDU at the end of Task 5.

9. The pretreatment solubilizes 20% of the cellulose, 95% of the xylose, 90% of the arabinose, SO% of the protein, and *55%* of the acetate. All of these values are based on PDU data. (Not all values were reached during Task 5, where pretreatment was less effective than desired.)

10. 91% of the starch is converted to fermentable form in pretreatment. All of this soluble material is converted to monomer by amylase in the **SSCF** process. The total conversion of starch is equivalent to that achieved in commercial corn-to-ethanol facilities.

11. Reactivity of the pretreated solids is "ideal,"
This reactivity is consistent with the
solubilization, as was achieved in the PDU tests (but not in Task 5).

12. Pretreated slurry is flashed to atmospheric pressure, cooling the **solids** and removing some inhibitors such as furfural. The amount of inhibitors removed is unknown; the inhibitor production during pretreatment is based on the inhibitors known in the SSCF feed in the PDU. The flashed liquids are condensed and treated *to* remove the inhibitors.

13. After flashing, the **slurry** is passed through a pump feeder and a positive displacement **pump.** In the pump feeder, slaked lime is added to raise the pH from 1.0 to 5.0, and the slurry is diluted from **34.1%** solids (PDU data) to the proper level to give 25% solids (soluble and insoluble solids) in the feed to the first SSCF reactor. The diluent is recycled fluid from the last SSCF stage (which contains ethanol) and thin stillage. PDU operation did not include the use of slaked lime (sodium hydroxide was **usually** used) **or** recycles.

Enzymes

15. Glucoamylase is purchased for $2.00/liter. The dosage used is equivalent to that used in the corn starch to ethanol industry, 0.10 ml/kg total solids. A much higher dose was used in the PDU runs, but the cost of this enzyme is less than $0.01/gallon of ethanol even at the higher dose.

Seed Fermenters

16. No seed fermenters are included in the design. Inoculum is added at the beginning of the continuous operation, and there is no need to add more yeast (PDU data shows that the yeast grows fast enough so that it is not washed out). The initial inoculum is grown in the first fermenter.

SSCF

17. The cellulose hydrolysis and fermentation of both glucose and xylose are carried out simultaneously in a series of stirred tank reactors. Each tank has a residence time of one day (24 hours). Residence time in the PDU was typically 36 hours per vessel.
18. The reactions are run at 30°C, pH = 5.0 and 5 psig. Heat of reaction is removed using external heat exchangers. pH is controlled using ammonia addition (the PDU used NaOH).
19. Corn steep liquor (CSL) is the primary source of nitrogen and other nutrients for the yeast. The yeast is LNHST-2, a recombinant yeast capable of fermenting both glucose and xylose (also galactose and mannose are fermented). CSL is added to the first reactor at a rate of 9.008 lb/gallon of feed to the SSCF train.
20. Cellulase enzyme is added. The addition of cellulase is not economically justified on the basis of increased yield of ethanol; it was added to provide a more useful platform case model for the sensitivity studies. The most economical case uses no cellulase addition.
21. Slurry in the tanks is mixed by the circulation of liquid through the external heat exchangers, and through the action of a agitator in each tank. Agitator power is 0.37 hp/1000 gallons. The PDU used jacketed vessels and large agitators, but the design applies current commercial practice from the corn to ethanol industry.
22. Ethanol concentration leaving the last SSCF vessel is 7%. The level is maintained by recycling part of the liquid product from the last SSCF vessel to dilute the incoming slurry.
23. Acetic acid level is at a low enough level to prevent significant inhibition of xylose fermentation.

24. Cooling water is used to remove heat of reaction for most of the year, but for the three hottest months, chilled water is needed.
25. The SSCF vessels run without contamination. The PDU runs were periodically contaminated, but commercial operation typically solves this problem.
26. Yields and conversions were calculated using the kinetics model developed during Phase 3. These yields and conversions are given in Table 4.1 for the platform case. The following were applied to this model:

R 1

. Lactic acid is a product of contamination. The level of lactic acid in CSL during Phase 3 was used; there was no lactic aid in the feedstock, or from contamination in the SSCF process. Hopefully, there will be no lactic acid if ammonia is used as the nutrient and contamination is avoided in SSCF.

c. It is assumed that xylitol, an intermediate reaction product of fermentation, will be consumed to yield ethanol in the commercial process. This was not usually accomplished in the PDU during Phase 3.

d. The spreadsheet model assumes that all glycerol and cell mass is derived from glucose. The kinetics model allows production of both from xylose as well. The source of these compounds has no significant effect on the results of the process model.

e. Cell growth in the kinetics model was adjusted to match the PDU results; growth in laboratory tests was significantly higher than in the PDU.

Tables 4.1 through 4.7 show the enzymatic cellulose conversions and xylose-to-ethanol fermentation yields used to establish the platform case.

Recycle Ethanol

27. A cross-flow filter is used to separate enough ethanol-rich liquid from the cooling loop in the last fermenter to maintain the ethanol concentration in the SSCF product at 7 wt%. Only enough liquid is separated to supply recycle. This concept was not tested in the PDU.

Distillation

28. Beer is fed to a still; the still design was supplied by Delta T Corporation. Molecular sieves are used to separate the azeotropic ethanol-water mixture. The ethanol is denatured with a sub-octane refinery stream (lower cost than regular gasoline) at 5% dosage.

Solid-Liquid Separation

29. Centrifuges or an improved cross-flow filter is used to remove water from the solids downstream of the still. The cake contains 35% solids. PDU cake contained on 25% solids, but tests by Alfa Lava1 showed 33-34% was possible.

30. The liquid from the solid-liquid separation is the thin stillage. Some of this liquid is recycled to reduce the solids in the pretreated feed. The rest of the Liquid is combined with other waste water and sent to anaerobic digestion. Anaerobic digestion of a similar stream was tested at NREL in Phase 2.

Solids Drying and Cooling

3 1. The centrifuged cake is sent to existing steam tube dryers. where the water content is reduced to 10%. Because the dryers and solids handling equipment are preexisting, they are not shown in the platform case material balance. Based on results of animal feeding tests using Task 4 and Task 5 product, steam tube drying damages the protein, and should not be used; possible alternatives are nozzle-disk centrifuges or spray drying.

32. Solids are sold based on their protein content. Protein is valued the same as that in corn fiber. Since the soluble protein is incorporated by the yeast during SSCF, the product solids have the same value as the feed, less minor process losses.

Utilities

32. Steam is purchased from existing equipment at $3.00/million BTU, electricity is purchased for $0.045/kWh, and water is purchased for $0.75/thousand gallons. Methane produced in anaerobic digestion is used in existing boilers and valued the same on a heating value basis as natural gas costing $2.85/million BTU. The only new utilities are the anaerobic digestion and chilled water systems.

Overall Design

33. On stream time is 350 days/year. Pekin actual operation is 365 days/year.

34. Manpower for the add-on plant is 11 employees.

35. There is no ethanol storage added beyond that already in place.

Results for the platform case optimization are given in Table 4.8. Cellulose doses evaluated vary between zero and 20 IFPU/g cellulose and residence time between one and eight days. Ethanol cost varied between $0.89 and $1.59/gallon. The platform case utilizes 5 IFPU cellulase/g cellulose and 72 hours residence time in three 950,000 gallon tanks. The case for zero enzyme dose and 24 hours residence time gives lower cost ethanol.

The resulting spreadsheet for the platform case assumptions is provided in Appendix A-3.2 and A-3.5. The first eight pages are material balances which show the flow rates and compositions of the various process streams. Pages one through eight are attached in Appendix A-3.2. Summary sheets and inputs/outputs for the platform case (pages nine through eighteen) are attached in Appendix A-3.5. Pages nine through twelve give most of the input parameter values including personnel and their salaries and cost of all chemicals in 1996 dollars. Pages thirteen and fourteen summarize recycles, mass, and enthalpy balances. Page fifteen shows the overall mass balance. Page sixteen shows all reactions stoichiometries, and page seventeen shows all physical properties of all components used in the spreadsheet. Page eighteen shows the utility summaries for all process areas.

4.4 Economic Evaluation - Spreadsheet Results for Platform Case

This section summarizes the economic evaluation of the platform case which was described in general in Sections 4.1 and 4.2, with more detailed design assumptions provided in Section 4.3. The detailed assumptions are important because they are central to the sensitivity analyses carried out on the platform case and described in Section 4.5

4.4.1 Platform Case Summary

The model is based on discounted cash flow analysis, using 1996 costs. The results of the spreadsheet model's economic evaluation is shown on the SWAN Cost Estimate Summary (Table 4.4.1). A 15% internal rate of return is assumed. In addition the model assumes that 20% contingency is added to the total installed cost. Other costs included are 4% construction management cost, 8% cost for engineering design, 2% cost of procurement, 3% freight cost on equipment, and 3% sales tax on all equipment. Maintenance, taxes, insurance and overheads (MTIO) are 3.20% of total initial capital per year. Costs for sitework and buildings are also included in the total capital.

4.4.1.1 Capital Cost

The capital cost in 1994 dollars based on Amoco/Flour Daniel equipment cost estimate (performed in 1991) is $47.5 million; about 13.5% of this cost is the royalty for the technology. This results in a cost per gallon of ethanol of $0.92/gal. Figure 4.2 shows the platform case net ethanol cost over the entire range of fermentation residence times and the enzyme dosages . The results of the spreadsheet model's economic evaluation are shown on the SWAN Cost Estimate Summary (Table 4.4.2) based on the new SWEC capital cost estimate completed at the end of 1996. The estimate, based on equipment cost estimates in 1996 dollars, is $41 .O million. These costs are defined in the attached capital cost summary table, and are both based on vendor quotes and SWEC's internal data base. All the capital cost estimates are accurate to about ±25%. Section A-3.4 of this report contains a more detailed discussion of the equipment and capital costs.

In both cases, an annual capital charge equal to 17.63% of the total initial capital cost is used. This is estimated by an internal Amoco accounting method and assumes a 15% internal rate of return. Plant life is assumed to be 15 years. However. the total capital is depreciated over 5 years.

The revised SWEC equipment capital cost estimate results in $0.06 reduction in cost of ethanol or a net cost of $0.86/gal. The overall capital charge per gallon of ethanol is $0.34.

4.4.1.2 Operating Cost

Operating costs consist of three major parts, variable costs such as raw materials and utilities, fixed costs such as labor,and MTIO, and byproducts credits. These are shown on the summary sheet and in more detail in the Square Case Economics Summary.

Variable Cost.

Variable cost includes feedstock costs, utilities costs, and chemicals and enzymes costs. Feedstock throughput is 750 dry tons per day at a cost of $0.20/lb of protein content and contributes $0.38/gal to the cost of ethanol. (The cost of feed is affected by the value of the solid coproduct, as shown under Byproduct Credits.) Chemicals used are sulfuric acid, lime, waste water chemicals, cooling water chemicals, corn steep liquor, and denaturant. Total chemicals costs contribute $0.14/gal to the cost of ethanol.
Glucoamylase adds insignificantly ($0.0003/gal) to the cost of ethanol. Utilities - steam, water, and electricity - add a total of $0.16/gal to the cost of ethanol. No natural gas was used in the base case. For more details on utility consumptions and their unit costs, see Section 4.3 above, or the attached Square Case Ecoonomics Summary from the spreadsheet.

Fixed Cost

This consists of the labor, MTIO and capital costs. The total number of personnel is 11 for this plant which adds $0.06/gal to the cost of ethanol. This cost includes salaries, overheads, productivity bonus, and training allowances. MTIO costs are assumed at 3.290 of the capital, which contributes $0.03/gal. Capital costs add an additional $0.39 to $0.34 per gallon, depending on which capital cost is used.

Byproducts Credits

This consists of credits for methane and animal feed coproduct. Methane is produced at a rate of about 58 MSCFH. Its credit (which is corrected for its actual heat content) is $0.05/gal of ethanol produced. Animal feed coproduct is produced at a rate of 336 dry tons/day and contains about 17.2% protein. The credit taken for the coproduct is $0.38/gal of ethanol produced. Thus, estimated total byproduct credit is about $0.43 per gallon of ethanol.

4.5 Sensitivity Study

During Phase 3 several design and operating parameters were identified as having potentially significant effects on the capital and operating costs for the conceptual design described above in section 4.3. This section reports on economic sensitivity studies in which both kinetics and operating variables were modified to examine their incremental effect on the cost of ethanol from the base case design. The primary purpose is to identify any work needed to justify the CRADA proceeding to Phase 4.

The analyses used the conceptual design's material balance and capital and operating cost estimates in SWAN's spreadsheet simulation as the platform case. The appropriate parameters were modified to represent the specific changes in kinetics and operating variables to be studied. A total of thirty seven effects were considered. The revised spreadsheet simulation results were then compared with the platform case results as reported below. It should be noted that the new SWEC capital cost estimate was not available in time, so all sensitivity case studies were based on the earlier Amoco/Flour Daniel capital cost estimate. Using the updated capital costs would not have a big impact on these results.

4.5.1 Discussion

The platform case was the basis for comparison for each effect studied. All parameters except those under consideration were held constant. Each run was optimized for residence time and cellulase dosage. Nineteen effects studied were kinetics related variables (see Table 4.10 for a complete list of these variables) and eighteen were other operating variables (see Table 4.11 for these variables).

Table 4.10. List of Kinetics Related Sensitivities

1. Base or Platform Case. See Table 4.8 and Figure 4.2 for the base case optimization - (using NREL Table 1741)
2. Base Case with batch fermentation.(effect of continuous fermentation versus batch - Table B 1743)
3. Base Case with no ethanol inhibition impact on the xylose fermentation. (effect of ethanol inhibition on xylose conversion - Table 2125)
4. Base Case with actual conversion of xylose in pretreatment. In the Base Case a target conversion of 95% is used. (effect of pretreatment conversion - Table 1549)
5. Base Case with 15% solids. (effect of lower solids - Table 1837)
6. Base Case with no xylose converted to xylitol as a byproduct. (effect of xylose conversion - Table 1789)
7. Base Case with no cell mass reduction. Base case assumes 2.083 g/liter reduction in cell mass in the first fermenter (effect of change in cell mass - Table 1765)
8. Base Case with measured starch conversion of glucans (effects of starch conversion and oligomeric glucans - Table 397)
9. Base Case with 100% starch conversion of glucans. (effects of starch conversion and oligomeric glucans - Table 421)
10. Base Case with all lactic acid removed. Base Case assumes only source of lactic acid is CSL. (effect of lactic acid - Table 1733)
11. Base Case with no lactic acid removed. (effect of lactic acid - Table 1749)
12. Base Case with actual conversions for enzymatic cellulose and xylose fermented to ethanol (effect of reactivity - Table 1737)
13. Base Case with no recycle (effect of recycle - Table 1743)
14. Base Case with no recycle and no acetic acid (Base Case acetic acid was removed) removal (effect of acetic acid inhibition on total ethanol production - Table 1744)
15. Base Case with measured conversions with 25% solids and no recycle (effect of actual PDU conversions versus targets - Table 20B)
16. Base Case with measured conversions with 15% solids and no recycle (effect of actual PDU conversions versus targets - Table 212B)
17. Base Case with measured fermentation residence time and enzyme dose for 25% solids (effect of actual PDU residence time and enzyme dose vs. optimum - Table 1741)
18. Base Case with measured fermentation residence time and enzyme dose for 15% solids. (effect of actual PDU residence time and enzyme dose vs. optimum - Table 1837)
19. Base Case with ammonia as nutrient. CSL was the nutrient in the Base Case. (effect of nutrient - Table 1733)
20. Synergistic effect of no cell mass reduction, ammonia as nutrient, conversion of all starch, no ethanol and no lactic acid inhibition to the Base Case. (effect of synergism - Table 1325A9)

All of the above sensitivities are summarized in Table 4.12. Optimization Table # 3 shows the sensitivity matrix with all NREL tables conditions, see appendix A4. All NREL Tables from 1741 to 1325 are also attached in appendix A4.

Table 4.11. List of Other Variables Sensitivity

1. Base or Platform Case. See item # 1 in Table 10.
2. Base Case using chilled water for twelve months. (effect of 12 months chilled water vs. 3 months - Table 1741)
3. Base Case not using chiller with fermenter temperature at 30°C. (effect of no chiller with xylose conversion for 12 months - Table 1741)
4. Same as item 3 except fermenter temperature at 30°C for about nine months and no xylose fermentation for about three months. (effect of no chiller with xylose conversion for 9 months - Table 1741)
5. Base Case with cellulase Make Option. (effect of manufacturing enzyme on site vs. purchasing enzyme - Table 1741)
6. Base Case with cellulase Buy Option (effect of lower enzyme cost - Table 1741)
7. Base Case with $4/bu of corn. (effect of feedstock cost - Table 1741)
8. Base Case with $6/bu of corn. (effect of feedstock cost - Table 1741)
9. Addition of evaporators. (effect of evaporators capital cost - Table 1741)
10. Solids in the filter containing 50% solids. Base Case assumes 35% solids. (effect of higher solids separation - Table 174 1)
11. Addition of dryers. (effect of dryer capital cost - Table 1741)
12. Addition of feedstock handling equipment. (effect of handling equipment capital cost - Table 1741)
13. Addition of boiler. (effect of boiler capital cost - Table 1741)
14. Addition of chemicals storage. (effect of chemicals storage capital cost - Table 1741)
15. Addition of full size cooling tower for entire plant. (effect of full size cooling tower capital cost - Table 1741)
16. Higher coproduct value, $100 per ton. (effect of higher coproduct value per ton due to e.g. higher protein content - Table 1741)
17. Lower coproduct value, $50 per ton. (effect of lower coproduct value per ton due to lower protein level or drying cycle degradation - Table 1741)
18. Lower plant capacity, 14.3 MM gallons of ethanol per year. (effect of lower feed rate on base case design, economies of scale - Table 1741)
19. Higher plant capacity, 42.8 MM gallons of ethanol per year. (effect of higher feed rate on base case design, economies of scale - Table 1741)

All of the above sensitivities are summarized in Table 4.14.

4.52 Kinetics Related Variables

Nineteen sensitivities to changes in the kinetics parameters were made. In each case, as single change was made to the kinetics model, and the tabulated output from that model was used to optimize enzyme dose and residence time in the commercial facility design Results are summarized in Table 4.12; the Run numbers listed below refer to the runs in Table 4.12.

Table 4.12

Target Conversions

STARCH	
Pretreatment	91.00%
CELLULOSE	
Pretreatment	20.00%
Enzymatic	%*
HEMICELLULOSE	
Xylan to Xylose	95.00%
Arabinan to Arabinose	90.00%
Acetate to Acetic Acid	55.00%
GLUCOSE	
Glucose to HMF	0.63%
Glucose to Fuse1 Oil	0.10%
Glucose to Cell Mass	%**
Glucose to Gly/Acet	2.509%
Glucose to Ethanol	88.00%
XYLOSE	
Xylose to Ethanol	%*
Xylsoe to Cell Mass	0.00%
Xylose to Furfural	0.95%

*These conversions change depending on fermentation holding time and cellulase dosage. Tables are used to obtain optimum conversions.

** This percent conversion is calculated by the model based on stoichiometries.

SD= 350 Cellulase Buy Option

Table 4.12 CRADA Phase Three Klnetlcs **Sensitivities Optimization** Amylase= 0.10 ml/Kg solid Solids @ SLS 35%

Chilled waler Is used for three months(with fermenters T_f=30 °C)

Item #	Effect Studied	NREL[2] Table	Recycle YIN	Max. EtOH %	Cellulase Buy Or Make	Cellulase Dose IFPU/liter	Resid. Time Hours	Number of Ferment?	Ferment'r Size MM Gal	Total Cap. Cost MM$	EtOH Prod. MMGPY	$/gal Net EtOH cost	Actual EtOH %	Cap. to Prod. Ratio	Diff. f'm Base EtOH Cost $/gal
1	Base/Platform Case[1]	1741A7.0	Y	7.0	B	5	72	3	0.95	47.50	21.39	0.92	7.00	2.22	-
2	Effect of Batch vs. Cont. ferm.	B-1743	N	N/A	B	5	48	4	0.95	52.10	2222	0.94	5.35	2.34	0.02
3	No EtOH Effect on Xyl. Ferm	2125A2	Y	9.0	B	5	46	2	0.95	43.70	21.66	0.87	8.64	2.02	-0.05
4	Effect of Actual pret. Xylan Conv.	1549A7.0	Y	7.0	B	5	72	3	0.95	46.80	19.91	0.98	7.00	2.35	0.06
5	Effect of lower solids, 15%	1837A7.0	Y	7.0	B	5	48	4	1.00	56.50	20.46	1.12	7.00	2.76	0.20
6	Effect of No Xylitol formation	1709A7.0	Y	7.0	B	5	72	3	0.95	47.70	21.68	0.92	7.00	220	0.00
7	Effect of No cell mass reduct.	1765A7.0	Y	7.0	B	5	72	3	0.95	48.20	22.30	0.90	7.00	2.16	-0.02
8	Effect of measured starch conv.	397A7.0	Y	7.0	B	5	72	3	0.95	4 5.90	1925	0.99	7.00	2.38	0.07
9	Effect of all glucans conv.	421A7.0	Y	7.0	B	5	72	3	0.95	48.50	22.79	0.89	7.00	2.13	-0.03
10	Effect of partial lactic acid inhibition	1733A7.0	Y	7.0	B	5	72	3	1.00	48.00	22.11	091	7.00	2.17	-0.01
11	Effect of Full lactic acid Inhibition	1749A7.0	Y	7.0	B	5	72	3	1.00	46.60	20.16	0.96	7.00	2.31	0.04
12	Effect of measured reactivity	1737A7.0	Y	7.0	B	5	72	3	1.00	46.30	18.40	1.94	7.00	2.52	0.12
13	Effect of No Recyck?	1743A7.0	N	N/A	B	5	72	3	1.00	40.90	21.74	0.94	5.23	225	0.02
14	Effect of Full acetic acid inhibit.	t744A7.0	N	N/A	B	5	96	4	0.95	51.40	2026	1.02	4.71	2.54	0.10
15	Effect of actual conv. 25% soilds	20-B	N	N/A	B	5	96	4	0.95	49.50	14.36	1.39	3.51	3.45	0.47
16	Effect 01 actual conv. 15% solids	212-B	N	N/A	B	5	72	5	0.95	59.40	15.62	1.50	2.27	3.75	0.58
17	Effect of NH_3 as nutrient	1733A7.0	Y	7.0	B	5	72	3	0.95	48.00	22.05	0.08	7.00	2.18	-0.04
18	PDU res. time & enzy. dose. 25%	1741A7.0	Y	7.0	B	7	108	5	0.85	53.40	23.05	1.01	7.00	2.32	0.09
19	PDU res. time & enzy. dose. 15%	1037A7.0	Y	7.0	B	7	108	12	1.00	76.90	23.31	1.32	7.00	3.30	0.40
20	Synergistic effect of all red. factors	1325A9.0	Y	9.0	B	5	48	2	0.95	44.30	23.11	0.80	927	1.92	-0.12

Note 1: This is the base or platform case with cellulase dose of 5 IFPU/g.
Note 2: NREL enzymatic cellulose hydrolysis and xylose fermentation Were used.

1. The following had a major impact on ethanol costs, increasing the cost by at least $0.03/gallon:
   A. Dilution of the solids fed to the first fermenter from 25% solids to 15% solids (Run 5). This is an alternative way to lower acetic acid concentration and thereby increase xylose fermentation rate -- increases ethanol cost $0.20/gallon over acetic acid removal assumed in the platform case.
   B. Using measured PDU Task 5 residence time (4.5 days) and enzyme dose (7 IFPU/g cellulose) raises the ethanol cost $0.09/gallon (Run 18). Running at 15% solids as well raises the cost $0.40/gallon above the platform case (Run 19).
   C. Using measured PDU Task 5 conversions:
      (1) Substrate reactivity was poor because of inadequate pretreatment -- increased ethanol cost by $0.12/gallon (Run 12).
      (2) Starch conversion was lower than in some other runs, increasing cost of ethanol by $0.07/gallon (Run 8).
      (3) Xylan conversion was low due to poor pretreatment -- increased costs by $0.06/gallon (Run 4).
   D. The model assumes both acetic and lactic acid inhibit fermentation. If acetic acid is not removed, ethanol cost will rise by $0.10/gallon (Run 14). If lactic acid is added at the rate observed in the corn fiber used in Task 5, and if contaminants in the SSCF process produce additional lactic acid as in Task 5, the ethanol cost would rise $0.04/gallon (Run 11).
   E. Using actual PDU performance (conversions and yields given in Table 4.13) in Task 5 would raise the ethanol cost $0.47/gallon at 25% solids feed (Run 15) and $0.58/gallon at 15% solids feed (Run 16).
2. The following had only a marginal (less than $0.03/gallon ethanol) impact on costs:
   A. Failing to convert the xylitol observed in Task 5 product liquid (Run 6).
   B. Running batch fermentations instead of CSTRs (Run 2).
   C. Producing as much cell mass as in laboratory experiments instead of the lesser amount in the PDU run (Run 7).
   D. Converting all of the starch in the feed to ethanol (Run 9).
   E. Adding in the lactic acid due to the feedstock in the PDU, but not that due to contamination (Run 10).
   F. Not using ethanol or thin stillage recycle (Run 13).

   Combinations of these effects can add up to a significant impact on costs.

3. The following had a major positive effect in lowering the cost of ethanol:
   A. Removing ethanol inhibition on xylose fermentation lowers the cost of ethanol $0.05/gallon (Run 3).
   B. Using ammonia in place of CSL as a nitrogen source lowers the cost of ethanol $0.04/gallon (Run 17).
   C. The combination of all positive effects -- higher cell mass, ammonia as the nutrient, total starch conversion, no ethanol or lactic acid inhibition -- would lower the cost of ethanol $0.15/gallon (Run 20).

The sensitivities to kinetics parameters show how important it is (1) to remove acetic acid and to keep lactic acid from getting into the process, (2) to pretreat the feed properly, and (3) to utilize ammonia (or other low cost nitrogen source) as the nutrient.

Table 4.13

PDU Conversions Based on Actual Data

	25% solids	15% solids
STARCH		
Pretreatment	77.00%	77.00%
CELLULOSE		
Pretreatment	20.00%	20.00%
Enzymatic	0.00%[1]	0.00%[3]
HEMICELLULOSE		
Xylan to Xylose	67.00%	67.00%
Arabinan to Arabinose	90.00%	90.00%
Acetate to Acetic Acid	55.00%	55.00%
GLUCOSE		
Glucose to HMF	0.61%	0.23%
Glucose to Fuse1 Oil	0.10%	0.10%
Glucose to Cell Mass	0.00%[2]	0.00%[2]
Glucose to Gly/Acet	2.89%	5.00%
Glucose to Ethanol	88.00%	88.00%
XYLOSE		
Xylose to Ethanol	0.00%[1]	0.00%[3]
Xylsoe to Cell Mass	0.00%	0.00%
Xylose to Furfural	2.84%	2.14%

Note (1): NREL Table 20-B which is generated from the actual 25% solids data is used to optimize the run for these conversions.

Note (2): These conversions are calculated based on the stoichiometries.

Note (3): NREL Table 212-B which is generated from the actual 15% solids data is used to optimize the run for these conversions.

4.5.3 Other Variables Sensitivity

Eighteen sensitivities were run on other operating and cost variables to see how ethanol cost was affected. The variables are listed in Table 4.11, and results of these studies are summarized in Table 4.14.

1. The following variables had no more than a marginal effect on ethanol cost:
   A. Price of corn -- because coproduct solid value is nearly equal to the feedstock value. the price of corn has little impact on ethanol cost (Runs 7 and 8).
   B. Use of chilled water to cool the fermenters for 12 months instead of 3 (Run 2).

C. Elimination of the chiller, either with or without an impact on xylose fermentation (Runs 3 and 4).

D. Adding capital for a boiler ($8.1MM, Run 13), an evaporator ($3.5MM, Run 9), or a solids dryer ($3.2MM, Run 11) raise the cost of ethanol by $0.11, $0.10, and $0.10/gallon, respectively.

Figure 4.3 (and Table 4.15 in Appendix A-3) show the rather significant impact of coproduct value on ethanol cost at four different protein levels. This plot assumes the cost of feedstock does not change. Figure 4.4 shows the impact of residence time and enzyme dose on ethanol cost. The lowest cost option is to use 10 IFPU enzyme and a residence time of two days. Figure 4.5 shows how ethanol cost varies with plant size; as expected, there are economies of scale.

The results of this set of studies show that (1) it is important to utilize existing equipment where it is available, (2) economies of scale are important, and (3) low cost enzyme can cut ethanol costs significantly. None of these observations was unexpected.

4.5.4 Ethanol Cost Variance Estimate

An estimate of variance for the net ethanol cost per gallon was developed. This was carried out to study the effect of uncertainties in the major input parameters. The case considered for this exercise was the actual PDU run with 25% solids. This case was selected since uncertainties in conversions have been identified by NREL during the Task 5 run. The following parameters with their associated variances were used:

1. Effect of variances in conversions for the 25% solids case. Positive and negative variances are outlined in Table 4.16.
2. Effect of variances in feedstock compositions for corn fiber and corn screenings. Composition variances are also outlined in Table 4.16.
3. Effect of variances in capital costs. The capital costs used in all cases are based on the original spreadsheet scaling factors. Variance of +25% and -25% was used as this is the expected accuracy of this estimate.
4. Effect of variances in solids recovery at the solid-liquid-separation (SLS). Base case assumes 35% solids recovery at the SLS. Based on the actual Solids Residue Recovery study performed during Task 5 run at the PDU, positive and negative variances were estimated for the solids recovery. Thus, a variance of ±5% for solids recovery was used.

SD= 350 Cellulase Buy Option

Amylase= 0.10 ml/Kg solid Solids @ SLS 35%

Chilled water is used for three months(with fermenters T_f=30 °C)

Item #	Effect Studied	NREL[2] Table	Recycle Y/N	Max. EtOH %	Cellulase Buy Or Make	Cellulase Dose FPU/liter	Resid. Time Hours	Number of Ferment?	Ferment; Size MM Gal	Total Cap, Cost MM$	EtOH Prod. MM GPY	$/gal Net EtOH Cost	Actual EtOH %	Cap. to Prod. Ratio	EtOH Cost Diff. f'm Base $/gal
1	Base/Platform Case[1]	1741A7.0	Y	7.0	B	5	72	3	0.95	47.50	21.39	0.92	7.00	2.22	-
2	Effect of Chiller for twelve monihs	1741A7.0	Y	7.0	B	5	72	3	0.95	47.50	21.39	0.94	7.00	222	0.02
3	Effect of No Chiller, Xyl. ferm. 12 mon.	1741A7.0	Y	7.0	B	5	72	3	0.95	44.30	21.39	0.89	7.00	2.07	-0.03
4	Effect of No Chiller, Xyl. ferm. 9 mon.	1741A7.0	Y	7.0	0	5	72	3	0.95	44.30	2139	0.94	7.00	2.07	0.02
5	Effect of Make Enzyme vs. Buy	t741A7.0	Y	7.0	M	10	48	2	0.95	46.50	23.30	0.78	7.00	2.00	-0.14
6		1741A7.0	Y	7.0	B	5	48	2	0.95	43.00	1920	0.79	7.00	224	
7	Effect of lower feedstock $4/bushel	1741A7.0	Y	7.0	B	5	72	5	0.95	47.50	21.39	0.92	7.00	222	0.00
8	Effect of lower feedstock $6/bushel	1741A7.0	Y	7.0	B	5	72	3	0.95	47.50	21.39	0.92	7.00	222	0.00
9	Effect of evaporators addition	174117.0	Y	7.0	B	5	72	3	0.95	51 .00	21.39	1.02	7.00	2.38	0.10
to	Effect of a beller solids sep. @ 50%	1741A7.0	Y	7.0	B	5	72	3	0.95	46.70	21.39	0.91	7.00	2.18	4.01
11	Effect of Dryers addition	1741A7.0	Y	7.0	8	5	72	3	D.95	50.70	21.39	1.02	7.00	2.37	0.10
12	Effect of Feed Handling Eq. addition	1741A7.0	Y	7.0	B	5	12	3	0.95	40.70	21.39	0.94	7.00	228	0.02
13	Effect 01 Boiler addition	1741A7.0	Y	7.0	B	5	72	3	0.95	55.60	21.39	103	7.00	2.60	0.11
14	Effect of Chemicals storage addition	1741A7.0	Y	7.0	B	5	72	3	0.95	48.20	21.39	0.93	7.00	225	0.01
15	Effect of Full size CT addition	174tA7.0	Y	7.0	B	5	72	3	0.95	48.60	2t.39	0.94	7.00	227	0.02
16	Effect of Coproduct Value, $100/Ton[3]	174tA7.0	Y	7.0	B	5	72	3	0.95	47.50	21.39	0.76	7.00	222	-0.16
17	Effect of Coproduct Value, $50/Ton[3]	1741A7.0	Y	7.0	0	5	72	3	0.95	47.50	21.39	1.03	7.00	222	0.11
18	Effect of lower capacity, 14.3 MM gal	I741A7.0	Y	7.0	B	5	72	3	0.95	34.90	1426	0.99	7.00	2.45	0.07
19	Effect of higher capacity, 42.8 MM gal	1741A7.0	Y	7.0	B	5	72	4	0.95	59.40	28.52	0.85	7.00	2.08	-0.07

Note 1: This is the base or platform case with cellulase dose of 5 FPU/g.

Note 2: NREL enzymatic cellulose hydrolysis and xylose fermentation were used.

Note 3: Base Case coproduct value is $70.19/Ton and contains 17.2% protein.

Table 4.15 CRADA Phase Three Base Case Optimization

SD= 350 Cellulase Make Option

Amylase= 0.10 ml/Kg solid Solids @ SLS 35%

Chilled water Is used for three months(with fermenters T_f=30 °C)

NREL[1] Table Number	Recycle Y/N	Cellulase Dose IFPU/liter	Cellulase Buy Or Make	Residence Time Hours	Starch % Conv'n	Max. % EtOH	Chilled Water Y/N	# of Ferment?	Ferment? Size MM gal	Total'" Cap. Cost MM $	EtOH Prod. MM GPY	$/gal Net EtOH cost	Actual Ethanol %	Cap. to Prod. Ratio
1741A5.5	Y	5	M	24	91.00	7.00	Y	1	0.95	40.40	16.52	0.82	6.54	2.45
1741A7.0	Y	5	M	48	91.00	7.00	Y	2	0.95	44.60	19.20	0.79	7.00	2.32
1741A7.0	Y	5	M	72	91.00	7.00	Y	3	0.95	49.10	21.39	0.78	7.00	2.30
1741A7.0	Y	5	M	72	91.00	7.00	N[2]	3	0.95	45.90	21.39	0.75	7.00	2.15
1741A7.0	Y	5	M	96	91.00	7.00	Y	4	0.95	52.60	22.62	0.80	7.00	2.33
1741A7.0	Y	5	M	144	91.00	7.00	Y	6	0.95	59.50	23.51	0.85	7.00	2.53
1741A7.0	Y	5	M	192	91.00	7.00	Y	8	0.95	65.50	23.81	0.91	7.00	2.75
1741A6.0	Y	10	M	24	91.00	7.00	Y	1	1.00	41.90	17.42	0.82	6.90	2.41
1741A7.0	Y	10	M	48	91.00	7.00	Y	2	0.95	46.50	20.30	0.78	7.00	2.29
1741A7.0	Y	10	M	72	91.00	7.00	Y	3	0.95	50.80	22.35	0.78	7.00	2.27
174tA7.0	Y	10	M	96	91.00	7.00	Y	4	0.95	54.50	23.36	0.80	7.00	2.33
1741A7.0	Y	10	M	144	91.00	7.00	Y	6	0.95	60.80	23.97	0.86	7.00	2.54
1741A7.0	Y	10	M	192	91.00	7.00	Y	8	0.95	66.90	24.09	0.92	7.00	2.78
1741A7.0	Y	15	M	24	91.00	7.00	Y	1	1.00	43.00	17.47	0.83	6.92	2.46
3741A7.0	Y	15	M	48	91.00	7.00	Y	2	0.95	48.00	20.91	0.79	7.00	2.30
1741A7.0	Y	15	M	72	91.00	7.00	Y	3	0.95	52.20	22.79	0.79	7.00	2.29
1741A7.0	Y	15	M	96	91.00	7.00	Y	4	0.95	55.80	23.63	0.01	7.00	2.36
1741A7.0	Y	15	M	144	91.00	7.00	Y	6	0.95	62.10	24.06	0.87	7.00	2.58
1741A7.0	Y	15	M	192	91.00	7.00	Y	8	0.95	68.20	24.13	0.94	7.00	2.83
1741A7.0	Y	20	M	24	91.00	7.00	Y	1	1.00	44.40	17.88	0.04	7.00	2.40
1741A7.0	Y	20	M	48	91.00	7.00	Y	2	0.95	49.30	2123	0.80	7.00	2.32

Table 4.15 CRADA Phase Three Base Case Optimization

SD= 350 Cellulase Make Option

Amylase= 0.10 ml/Kg solid Solids @ SLS 35%

Chilled water is used for three months(with fermenters T_{ft}=30 °C)

NREL[1] Table Number	Recycle Y/N	Cellulase Dose iFPU/liter	Cellulase Buy Or Make	Residence Time Hours	Starch % Conv'n	Max. % EtOH	Chilled Water Y/N	# of Ferment'r	Ferment'r Size MM gal	Total[3] Cap. Cost MM $	EtOH Prod. MM GPY	$/gal Net EtOH Cost	Actual Ethanol %	Cap. to Prod. Ratio
1741A7.0	Y	20	M	72	91.00	7.00	Y	3	0.95	53.40	22.94	0.80	7.00	2.33
1741A7.0	Y	20	M	96	91.00	7.00	Y	4	0.95	57.00	23.70	0.82	7.00	2.41
1741A7.0	Y	20	M	144	91.00	7.00	Y	6	0.95	63.30	24.07	0.89	7.00	2.63
1741A7.0	Y	20	M	192	91.00	7.00	Y	8	0.95	69.30	24.12	0.96	7.00	2.87
1741A5.5	Y	0	M	24	91.00	7.00	Y	1	1.00	38.10	13.98	0.89	5.72	2.73
1741A6.0	Y	0	M	48	91.00	7.00	Y	2	1.00	41.90	15.44	0.89	6.32	2.71
1741A6.5	Y	0	M	72	91.00	7.00	Y	3	1.00	45.50	16.55	0.91	6.78	2.75
1741A7.0	Y	0	M	96	91.00	7.00	Y	4	1.00	49.00	17.35	0.93	7.00	2.82
1741A7.0	Y	0	M	144	91.00	7.00	Y	6	1.00	56.10	18.39	1.00	7.00	3.05
1741A7.0	Y	0	M	192	91.00	7.00	Y	8	0.95	61.10	10.63	1.07	7.00	3.28

Note 1: NREL enzymatic cellulose hydrolysis and xytose fermentation were used
Note 2: No chilled waler reduces the ethanol cost by about $0.03/gal.
Note 3: SLS presses costs were added to these runs.

Table 4.16

Actual PDU Conversions with 25% Solids

	Modified NREL	Negative[4]	Positive Variance[4]
STARCH			
Pretreatment	77.0%	67.0%	87.0%
CELLULOSE			
Pretreatment	20.0%	10.0%	30.0%
Enzymatic	0.0%[1]	0.0%[1]	0.0%[1]
HEMICELLULOSE			
Xylan to Xylose	67.0%	57.0%	77.0%
Arabinan to Arabinose	90.0%	90.0%	90.0%
Acetate to Acetic Acid	55.0%	55.0%	55.0%
GLUCOSE			
Glucose to HMF	0.63%	0.63%	0.63%
Glucose to Fuse1 Oil	0.10%	0.10%	0.10%
Glucose to Cell Mass	9.0%[2]	0.0%[2]	0.0%[2]
Glucose to Gly/Acet	2.50%	2.50%	2.50%
Glucose to Ethanol	88.0%	85.0%	90.0%
XYLOSE			
Xylose to Ethanol	0.0%[3]	0.0%[1]	0.0%[1]
Xylose to Cell Mass	0.0%	0.0%	0.0%
Xylose to Furfural	0.95%	0.95%	0.95%

Note 1: Optimized per NREL Table 20B, variances -30% and +30% were used.

Note 2: Calculated based on stoichiometries.

Note 3: Optimized per NREL Table 20B, variances -20% and +20% were used.

Note 4: All variances were provided by NREL.

Corn fiber and corn screenings feedstock has the following composition, biomass 45.7% and 54.3% water, Variances were estimated based on actual corn fiber and corn screenings blend. Biomass Composition:

Based on the estimated uncertainties stated above, sensitivities on the net ethanol cost was estimated and summarized in Table 4.17. Estimated variances in the ethanol cost are -$0.47/gal (or -34%) to +$0.84/gal (or +60%) compared to the optimized case of $1.39/gal of ethanol. See Table 4.10 for the optimum case using NREL Table 20-B. Based on the estimated variances obtained for net ethanol cost from the actual PDU data using 25% solids case, it is reasonable to assume that the variances on all cost of ethanol including the base case would also be in the same range of -34% to +60%. To apply this estimated variance to the base case of $0.92/gal the negative variance results in an ethanol cost of $0.61/gal and the positive variance results in an ethanol cost of $1.47/gal. Thus the base case ethanol cost/gal range is, $0.61 < $0.92 < $1.47. It should be noted that if the estimated variances were all ±25% the variance range in the base case ethanol cost/gal would be $0.69 < $0.92 < $1.15.

Table 4.17

NREL Mod&dative	Variance		Positive Variance
Starch	27.20%	23.94%	30.90%
Cellulose	15.60%	13.73%	17.72%
Xylan	16.40%	14.44%	18.63%
Arabinau	10.60%	10.60%	10.60%
Acetate	4.04%	4.04%	4.04%
Lignin	7.40%	13.07%	7.40%
Protein	7.50%	6.60%	8.52%
Insol Ash	0.70%	0.70%	0.70%

4.5.5 **Conclusions**

The study of sensitivities was lopsided in that many more of the effects examined had a negative impact on costs than a positive one. This is not uncommon when new processes are evaluated because it is generally easier to see what can go wrong than to envision reasonable improvements. Most improvements are usually already included in the platform case.

The sensitivity studies showed the following:

1. Task 5 in the PDU is not a good model for a commercial facility. Pretreatment was poor, and acetic acid reduced ethanol yields. Costs for a plant operating under these conditions would be unacceptably high.
2. There is some concern because the variance of the parameters yields a large degree of uncertainty into the prediction of commercial costs for ethanol. Efforts are needed both to reduce uncertainties in PDU measured variables and to more carefully estimate capital and operating costs. However, the degree of precision in the engineering costs seems appropriate for the current level of understanding of the process.
3. The following must be demonstrated before it is prudent to proceed to Phase 4 under the CRADA:

    a. Optimum pretreatment of corn fiber/corn screenings must be demonstrated in the APR.

    b. Coproduct solids must be properly dried and successfully tested as an animal food. The value of the coproduct is critical to the overall ethanol economics for this feedstock.

4. Two improvements could reduce the cost of ethanol, but are not as critical to those above:
   a. Improve the fermentive organism to react xylose to ethanol more quickly when acetic acid (for lactic acid) and ethanol are present.
   b. Discover why starch conversion to ethanol was incomplete in some PDU runs, and identify how to avoid this problem.
   c. Ammonia should be proven in the PDU as a source of nitrogen for the fermentive organism. This will both reduce the cost of the nutrient and help keep lactic acid out of the SSCF vessels. It is expected that if ammonia works for any feedstock, it should work for corn fiber; the reverse may not be true, since corn fiber contains all necessary nutrients other than nitrogen.

5.0 Recommendations for Future Work

The review of the experimental results presented in Section 2 and 3 and results of the process design and economic analysis of the proposed process presented in Section 4, lead to the conclusion that the ethanol from corn fiber process developed under the CRADA is not yet ready for operation in a demonstration plant. The reasons are both technical and economic. There are five research areas, described below, that have emerged as being critical to improving the currently defined process.

5.1 Pretreatment

Identifying and controlling pretreatment conditions and therefore performance are critical to understanding fermentation performance. During Phase 3 it was not possible to achieve known and repeatable conditions in the APR.
The changing pretreatment severity has made it difficult to compare bench fermentation data to PDU data.

Effort is needed to understand all aspects of APR operation, including appropriate measurements and controls, so that performance can be predicted and repeatable conditions can be routinely achieved. Satisfactory mass balance closure around the APR has not been demonstrated, however this is precluded to a large degree by the inability to tightly control performance.

Substrate reactivity was poor during much of the Phase 3 work because of inadequate pretreatment. A sensitivity study looking at the substrate reactivity measured during Task 5 resulted in an ethanol cost increase of $0.12/gallon compared to what was deemed to be acceptable reactivity in the platform case. Two other sensitivity studies related to measured Task 5 pretreatment performance showed that: (1) starch conversion was lower than in some other runs, increasing cost of ethanol by $0.07/gallon, and (2)xylan conversion was low due to poor pretreatment, increasing costs by $0.06/gallon. All of these sensitivity studies show how important it is to pretreat the feed properly.

If improved repeatability proves to be the case with tests on rice straw, then additional work associated with the APR under the CRADA should be done in concert with the equipment scaleup activities. This way any experiments would serve the dual purpose of providing important process information that is also consistent with data required for scaleup.

5.2 Unconverted Sugars

A significant loss of potential ethanol is leaving the process as unconverted sugars. Converting these sugars (primarily cellulose, oligomeric glucose, and monomeric and oligomeric xylose), which represent approximately 50% of the total fermentable sugars, to ethanol may be necessary to achieve an economical process. Work is needed to identify and then convert the oligomeric glucose (if this is what it truly is) to a fermentable form. It is also important to convert more of the xylose (oligomeric and monomeric) to ethanol.

The conversion of oligomeric xylose during the latter half of the Task 5 run is promising and should be investigated as a way of converting additional xylose to ethanol. From a modeling perspective, kinetic expressions that describe the utilization of oligomeric sugars would improve the overall process model.

If lower solids concentrations are required, the trade off between lower solids concentration versus additional xylose conversion needs investigation. This was partially investigated with sensitivity studies at different solids concentrations. The results indicate that dropping the solids concentration from 25% to 15% for the platform case (all other variables held constant) increases ethanol costs by $0.20/gallon. Other sensitivity analyses using actual PDU performance in Task 5 raises the ethanol cost $0.47/gallon at 25% solids feed and $0.58/gallon at 15% solids feed above the cost of the platform case.

A secondary but related need is a better understanding of what controls fermentable sugar diversion to byproducts such as cell mass, glycerol and xylitol so that yields can be described as functions of other components, rather than just constants. Sensitivity studies indicate that taken separately these effects have a small effect on the cost of ethanol production. Collectively, however, the impact would be more significant. Nancy Ho has suggested that anaerobic operation may prevent xylitol formation. Many of the fermentations cart-tied out during Phase 3 allowed the introduction of air, either passively as in shake flasks, or purposefully as in overlay air for the PDU fermenters

5.3 Fermentation Performance

Experimental data from Phase 3 has shown that inhibitors, such as ethanol, acetic acid, lactic acid, HMF and furfural, significantly reduced xylose conversion. At 25% solids, xylose conversion is only 25%, but increases to near 75% when solids concentration is lowered to 15% (effectively diluting the inhibitors). However, this produces higher capital and operating cost for the plant. The sensitivity study which examined the effect of diluting of the solids fed to the first fermenter from 25% solids to 15% solids showed that ethanol cost increases $0.20/gallon over acetic acid removal assumed (at zero cost) in the platform case.

The kinetic model assumes both acetic and lactic acid inhibit fermentation. Acetic acid is a natural byproduct of the pretreatment step, as it is derived from acetyl groups naturally occuring in biomass. Some feedstocks, such as corn fiber, have relatively high acetyl concentrations and may therefore always result in high acetic acid levels when pretreated. If acetic acid is not removed, ethanol cost will rise by $0. 10/gallon. If lactic acid is added at the rate observed in the corn fiber used in Task 5, and if contaminants in the SSCF process produce additional lactic acid as in Task 5, the ethanol cost would rise $0.04/gallon. These sensitivities to kinetics parameters show how important it is to remove acetic acid and to keep lactic acid from getting into the process.

Improving organism performance in the presence of higher inhibitor concentrations could also improve process economics. Approaches to solving this problem could involve bench scale efforts designed to remove the inhibitors, or microorganism adaptation or mutagenisis to tolerate higher levels of inhibitors. NREL is currently prohibited from making improvements to the organism under the Material Use Agreement with Purdue.

Understanding ethanol inhibition is of primary importance. Application of processing strategies to manipulate ethanol concentrations during the fermentation stage will depend on quantitative effects of ethanol on LNHST2 performance. Ethanol recycle could have promise if it is determined that ethanol tolerance is not a problem. A sensitivity study to evaluate removing ethanol inhibition on xylose fermentation lowers the cost of ethanol $0.05/gallon.

Examining nutrients as a factor limiting tolerance of LNHST2 to inhibitors is another possibility. Limitations on high ethanol yeilds in brewing yeasts have been shown to be due to a nutritional deficiency rather than ethanol toxicity. Very little is known about the quantitative effects of nutrients on LNHST2 performance, particularly when inhibitors are present.

5.4 Coproduct Recovery, Processing and Quality

Solids remaining after fermentation are recovered and sold for their value as animal feed. It is expected that the value will be based on the protein content of the material. The fate of protein and important amino acids need to be tracked though the various processing steps to determine how to maximize coproduct yield and value. Figure 4.3 showed the rather significant impact of coproduct value on ethanol cost at four different protein levels.

Solid-liquid separation equipment as well as solids drying equipment are required. Centrifugation was tested in the PDU and by Alfa-Laval Sharples and Bird Machine Company. The tests indicated that there is a significant tradeoff between high percent solids in the wet cake and solids recovery levels. None of the centrifuge configurations tested gave adequate results from a solids recovery and cake concentration perspective. Centrifuging the whole stillage at higher temperatures should be looked at to improve separation of centrate from the wet cake. Rotary vacuum filtration is not practical for this application based on vendor tests. Therefore, alternative techniques need to be investigated for separating whole stillage to maximize recovery and minimize costs, including the drying costs.

Coproduct drying tests were carried out in rotary steam tube dryers. Results indicate that although this type of equipment can be used, there are indications that the rotary dryers may downgrade the product by darkening it and possibly reducing its nutritional value. The cost estimate assumed that an existing solids dryer would be used. However, if a new solids dryer was required it would raise the cost of ethanol by $0.10/gallon. In addition, drying the coproduct in this manner results in a fine powder which must be subsequently pelletized before it can be used in the animal feed market. This cost was not included in the cost estimate. Alternatives to be investigated include flash drying and spray drying. Process designs could be carried out however, tests should be conducted to evaluate these forms of drying on coproduct quality.

Animal feeding tests were carried out on poultry, swine and cattle. Unfortunately, final test results are not available yet. These studies need to be completed, and the results included in the technoeconomic evaluation.

5.5 Contamination

Contamination proved to be a recurring problem throughout PDU runs. The corn-to-ethanol industry has the advantage of operating at high ethanol concentrations (10%12%) which discourages most bacterial contaminants. Even then, doses of antibiotics are occasionally needed. High ethanol concentrations are detrimental to xylose conversion and even with adaptation of the organism, ethanol concentrations as high as the corn-to-ethanol industry are not likely to be achieved. This leaves the process vulnerable to contamination problems, particularly since the cost of aseptic designs are high and continual use of an antibiotic would also be costly.

Effective contamination control strategies are needed and may involve a combination of process parameters (temperature, pH, ethanol concentrations, antibiotics, etc.) and attention to design to avoid as many trouble spots as possible. However, it may be necessary to operate at a much larger scale than the NREL PDU to truly assess the magnitude of the contamination problems and determine effective control strategies.

5.6 Summary

Although many possible process improvements are possible, only a **few** are critical to developing a commercially attractive process. The critical items are (1) **prove** that direct steam injection solves the problems encountered with erratic APR operation, (2) find a solution to the inhibition of xylose fermentation by acetic acid, **(3)** develop suitable means for separating the solid coproduct from product slurry, **and** (4) demonstrate CRADA technology in a continuous, integrated PDU run.

Most of this work can be carried out on any feedstock. Some of it will undoubtedly be carried out under the Gridley Rice Straw Project. **Before** the **CRADA** process can be applied to corn fiber, solutions for the various problems must be developed, then there must be a continuous, integrated PDU **run** to demonstrate that these solutions work on corn fiber feedstock.

Two recommended tests that are only needed if corn fiber is the feedstock of choice are the optimization of corn fiber pretreatment, and the testing and validation of the solid coproduct as an animal feed. The solids needed for **the** animal feed test would be produced during the required integrated PDU run on corn fiber, and the amount of solids needed would determine the required *extent* **of** that ***run.***